Diatom communities across a gradient of acid mine drainage on the West Coast, South Island, New Zealand

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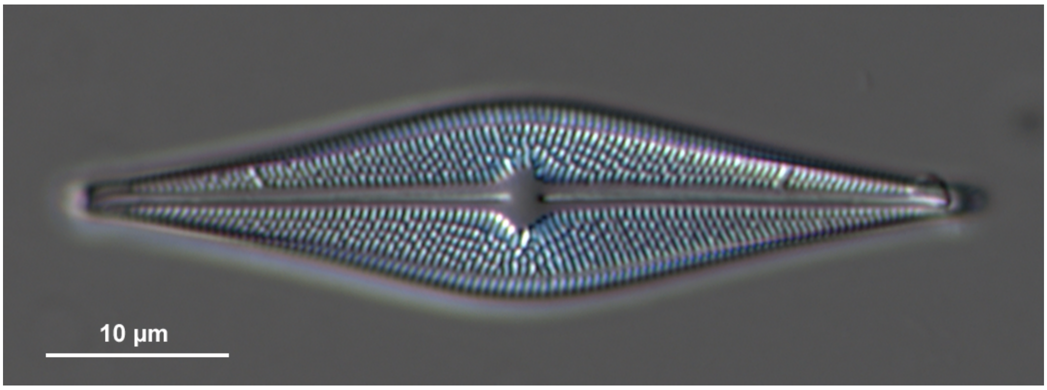

Light micrograph of *Brachysira serians* var. *acuta* collected from Conns Creek.

Abstract

Acid mine drainage (AMD) is a major environmental issue worldwide. On the West Coast of the South Island, New Zealand, numerous catchments receive AMD, with significant negative impacts on in-stream flora and fauna. Diatoms are commonly regarded as powerful biological indicators and may be found in high abundance in AMD-contaminated streams; however, relatively little work has been done on diatoms in mining environments in New Zealand. Initially, I conducted a survey of epiphytic diatom communities in 39 streams ranging from non-impacted reference streams to those severely impacted by AMD. Streams were assigned to one of four classes along an AMD gradient: circum-neutral reference, naturally acidic reference, moderately impacted, and severely impacted. There was a wide range in diatom taxonomic richness in reference and moderately impacted streams (8 – 33 taxa). Taxonomic richness was greatly reduced in severely impacted streams (1 – 5 taxa) at a threshold of pH 3.4 and was dominated by *Pinnularia* cf. *acidophila* (69 – 100% relative abundance). Community composition differed between circum-neutral reference, moderately, and severely impacted streams; however, naturally acidic and moderately impacted streams had similar diatom communities primarily composed of acid-tolerant *Eunotia* and *Frustulia* species. This indicated that diatoms are strongly structured by pH and able to tolerate moderate conductivity and metal concentrations without a corresponding shift in community composition. Survey data were then used to develop two diatom-based indices for streams impacted by AMD: a single Biotic Index and a Multimetric Index. While neither index was able to distinguish naturally acidic from moderately impacted streams, both indices successfully categorised streams as circum-neutral reference, moderately or severely impacted by AMD. These indices may be useful in assessing AMD impact on circum-neutral streams or in identifying when a stream has crossed a threshold from moderately to severely impacted by AMD. Diatoms would be especially useful as bioindicators of AMD if they respond rapidly to a change in mine discharge. To test this, mature algal biofilms were reciprocally transferred between circum-neutral reference streams and streams of varying degrees of AMD over a period of 13 days. Diatom mortality increased rapidly in the reciprocal transfer between reference and severely impacted streams. Reference communities resembled the ambient diatom community of severely impacted streams 13 days post-transfer. However, in the reverse transfer, a change in community composition was slow to occur. Diatoms respond faster to an increase in pollution than to pollution amelioration. Overall, results indicated that diatom communities may be a useful tool for monitoring the presence and magnitude of AMD in New Zealand streams.

Acknowledgements

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Chapter 1

Introduction

1.1. Coal mining in New Zealand

Global consumption of fossil fuels, including oil, gas and coal, is on the rise. As a result of increasing demand and diminishing reserves, coal is projected to last another 107 years, outlasting oil (35 years) and gas (37 years) (Shafiee and Topal 2009). If these estimates prove correct, coal will be the only available fossil fuel after 2046. Mineral-rich nations such as New Zealand will therefore increasingly rely on coal extraction to meet rising demand and to supplement renewable sources of energy for the foreseeable future (MED 2011a).

Coal mining has a long history in New Zealand. Coal was first discovered on the West Coast of the South Island in the 1860s, and mining began shortly thereafter (Harding and Boothroyd 2004). There are currently 20 operating coal mines in New Zealand, occurring in the Waikato and Taranaki regions of the North Island, and Southland, Otago and the West Coast of the South Island (MED 2011b). The West Coast has the largest reserves of high-quality bituminous coal, which is of high value to the international market due to its low ash content (MED 2011b). Coal is extracted through both underground and opencast mining methods. Historically, underground mining was prevalent; however, the amount of coal produced from opencast mining surpassed that of underground mining in 1970, and has since become the dominant extraction method (MED 2011c). In 2010, New Zealand produced 5.3 million tonnes of coal, and there are an estimated 15 billion tonnes of coal remaining in-ground (MED 2011b). Solid Energy, New Zealand's largest coal producer, recorded a net profit of $87.2 million in 2011 (Solid Energy 2011a). Coal mining has been, and will continue to play, a major role in local economic development, as well as the national economy. As the human population grows, the pressure placed on mineral extraction will continue to rise. The demand for coal is projected to increase by 65% over the next 20 years (IEA 2011). In response to increasing demand, Solid Energy has recently proposed two new mines for development within the Upper Waimangaroa catchment: the Cypress Mine and the Mt. William North Mining Project (Solid Energy 2011b).

Despite the obvious economic benefits of mining, the environmental consequences to both terrestrial and aquatic environments are well known (Palmer et al. 2010). With the expansion of coal mining, both globally and within New Zealand, comes the need to better understand the impact on the local environment. One of the most significant environmental consequences associated with current and historic coal mining is acid mine drainage (AMD) (Kelly 1988).

1.2. Acid mine drainage and its biotic effects

On the West Coast of the South Island mining occurs primarily within Brunner Coal measures that are rich in pyrite and sulphates (Pope et al. 2010). When sulphide-bearing coal is exposed to air and water, a series of chemical reactions take place (Akcil and Koldas 2006), resulting in the production of sulphuric acid and leaching of associated heavy metals into the surrounding environment. Streams receiving AMD within this region are often characterised by low pH (commonly pH < 3), high conductivity, and elevated concentrations of heavy metals, notably aluminium (Al), iron (Fe), zinc (Zn) and manganese (Mn). At low pH (< 5), Al becomes extremely soluble, and hence more toxic to aquatic flora and fauna (Planas 1996, Harding and Boothroyd 2004). In addition to the chemical effects of AMD, there may also be changes to the physical environment. As pH increases either by dilution downstream or near the confluence of a stream of higher pH, precipitates of metal hydroxide cover the streambed (Fig. 1.1A). In New Zealand, the most common metal precipitate associated with AMD is iron hydroxide ($Fe(OH_3)$). Iron precipitates from solution at pH 3.5 – 4.3 (Kelly 1988). In severely impacted streams where pH is below 3.5, iron hydroxide does not precipitate and the water appears clear (Fig. 1.1B). AMD is generated from both active and abandoned mines and can continue to impact waterways decades after mining operations have stopped.

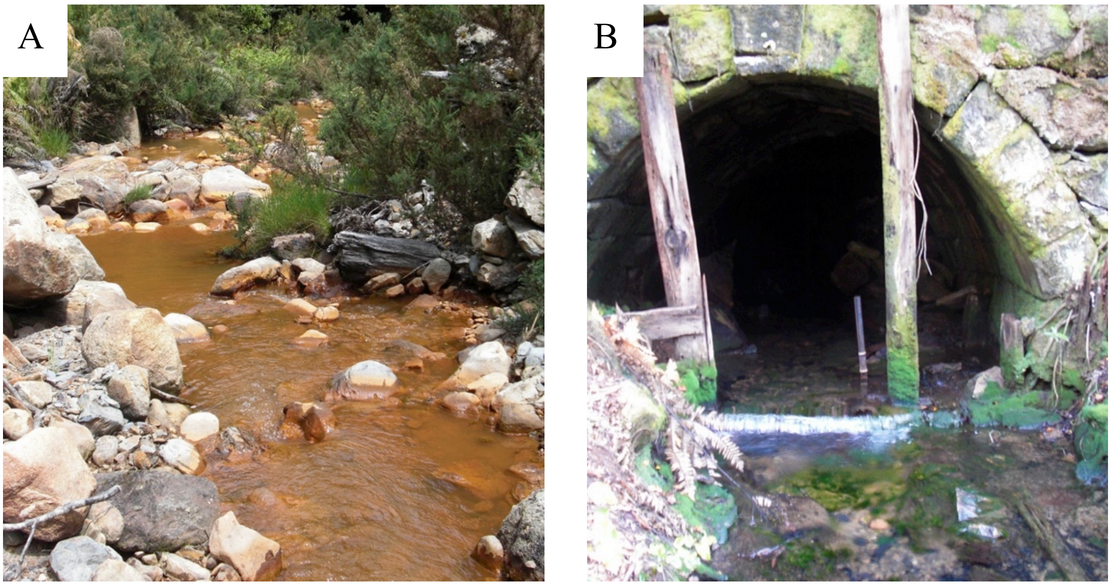


Figure 1.1. Garveys Creek (pH 4.3) with severe iron hydroxide deposition (A), and Bath House (pH 2.1), an abandoned adit with no visible iron hydroxide (B).

The chemical and physical changes associated with AMD have significant implications for aquatic flora and fauna (Hogsden and Harding 2012a), including deformities (Luís et al. 2011), decreased abundance (Simmons et al. 2005), and mortality (Gerhardt et al. 2008). While benthic macroinvertebrates can be found in AMD streams of pH < 3.6 (Winterbourn 1998), they are typically present in extremely low densities (Harding and Boothroyd 2004). Fish are often completely absent from severely impacted AMD streams (Greig et al. 2010, Hogsden and Harding 2012a). In contrast, algae may be present at high biomass, although typically restricted to a small number of acid-tolerant species (Harding and Boothroyd 2004, Bray et al. 2008). For example, in the U.S., Niyogi et al. (1999) found a single species of *Ulothrix* comprised nearly 100% of periphyton biomass in an AMD-contaminated stream of pH < 4. In New Zealand streams severely (e.g. pH < 3.5) impacted by AMD, species such as *Klebsormidium acidophilum* P.M. Novis and *Euglena mutabilis* F. Schmitz dominate (Bray et al. 2008). Thick mats of filamentous algae can be found in abandoned adits, possibly due to a combination of stable flow (Harding and Boothroyd 2004), lack of grazing pressure (Planas 1996), and a reduction in inter-specific competition as less-tolerant species are eliminated from the ecosystem (Planas 1996). While algae often proliferate in severely impacted streams of low pH and high metal concentrations, biomass may be restricted by the rate of metal oxide deposition. When algae are unable to grow through, or form a mat over, precipitates they are typically found at extremely low biomass (Niyogi et al. 1999) or are absent (McKnight and Feder 1984).

1.3. Diatoms in streams receiving AMD

Monitoring of AMD-contaminated streams within New Zealand has historically focused on water chemistry, and more recently benthic macroinvertebrates (Pope et al. 2005, Gray and Harding in press). In the Northern Hemisphere, algae, especially diatoms, are frequently employed to assess the presence and magnitude of AMD (Verb and Vis 2005, Smucker and Vis 2009). Several previous studies have found that diatom communities within AMD streams are significantly different from those that inhabit reference streams, and comprise primarily acidophilic and acidobiontic species (Verb and Vis 2000, Zalack et al. 2010). The genera *Eunotia*, *Frustulia, Nitzschia* and *Pinnularia* are particularly well represented in acidic environments (DeNicola 2000). The most commonly observed species in AMD streams is the cosmopolitan species *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst (DeNicola 2000, Smucker and Vis 2009, Zalack et al. 2010). Acidophilic *E. exigua* has been found in environments with pH as low as 2 (Lessmann et al. 2000). Van Dam et al. (1981) consider *E. exigua* to be the most metal-tolerant of all diatom species, further facilitating its prevalence in AMD-contaminated streams.

In addition to changes in community composition, several previous studies have identified a decrease in diatom taxonomic richness and diversity in streams impacted by AMD (Verb and Vis 2000, Niyogi et al. 2002, Luís et al. 2009). For example, Zalack et al. (2010) found richness was significantly negatively correlated with pH ($r = -0.71$), specific conductivity ($r = -0.58$) and Fe ($r = -0.64$). Similarly, Niyogi et al. (2002) identified a significant negative relationship between algal diversity and AMD stress. The stress of low pH, high metal environments selects for a small subset of tolerant species from the regional pool. However, the relationship between richness and an AMD contamination gradient is not always linear. Diversity and richness may be higher in moderately impacted streams than in reference streams (Smucker and Vis 2009). In moderately stressed systems, additional factors that may influence diatom diversity include light intensity, flow rate, and nutrient concentrations (Verb and Vis 2000).

Understanding the complex interaction between acidity and heavy metals is a challenge often faced by researchers (Hogsden and Harding 2012a). Each in turn may impact the diatom community in different ways. For example, heavy metals have been shown to correlate with a decrease in frustule size (Corcoll et al. 2012) as well as increase the occurrence of deformities resulting in teratological forms (Falasco et al. 2009, Ferreira da Silva et al. 2009). While metal-pollution alone impacts diatom diversity and community composition (Hill et al. 2000, Gold et al. 2002), pH is often cited as the dominant environmental factor in structuring the diatom community at a site, both in reference (Pan et al. 1996) and AMD streams (Verb and Vis 2005, Bray et al. 2008).

Diatoms have long been recognised as accurate indicators of current and historic acidity in both lotic and lentic systems (Battarbee et al. 2010). The earliest pH classification system was proposed by Hustedt (1937-1939), who assigned diatom species to one of five classes: alkalibiontic, alkaliphilous, indifferent, acidophilous, or acidobiontic. While this system is still used today (with an additional 'circum-neutral' category), it is now recognised that very few taxa are actually indifferent to pH. Of the 948 taxa included by van Dam et al. (1994), only one, *Eunotia bilunaris* (Ehrenberg) Schaarschmidt was classified as pH indifferent.

Algae living in extremely acidic environments have several unique physiological traits that allow them to tolerate these conditions. For example, they are able to maintain a near neutral internal pH despite living in an acidic medium. This is due to the relative impermeability of the plasma membrane for protons, which requires the cell to exert little energy for active transport (Gross 2000). In addition, pH influences the availability of dissolved inorganic carbon (DIC), which is a requirement for photosynthetic organisms. At pH < 4, bicarbonate and carbonate are converted into carbonic acid (Harding and Boothroyd 2004). As an adaptation to low levels of DIC, algae may grow in near terrestrial environments to enhance the rate of CO_2 diffusion, or motile algae may move to areas of high CO_2 (Gross 2000).

1.4. Diatoms in naturally acidic streams

Studies on the relationship between benthic diatoms and acidity primarily focus on anthropogenically acidified systems (e.g. Verb and Vis 2000, Bray et al. 2008, Zalack et al. 2010). In addition to AMD-contaminated streams on the West Coast of New Zealand, a number of catchments also have naturally acidic, brown water streams (pH 4.3 – 5.7). The natural acidity is produced by the leaching of fulvic and humic acids from decomposing vegetation and soil (Collier et al. 1990). The effect of natural acidity on stream biota is thought to be less severe than that of more recent anthropogenic acidity, as the flora and fauna of naturally acidic streams have had time to evolve and adapt to current conditions (Collier et al. 1990, Greig et al. 2010). In addition, metal concentrations are typically either much lower than those of AMD-contaminated streams, or metals are bound to organic matter and made unavailable to biota (Collier et al. 1990, Sparling and Lowe 1996). Naturally acidic streams provide a rare opportunity to investigate the effect of low pH on aquatic organisms in the absence of the confounding effects of heavy metals.

The flora and fauna of naturally acidic lotic ecosystems have not been well studied. Overseas literature indicates that naturally acidic streams support a diverse diatom community, typically dominated by *Eunotia* species (Passy et al. 2006, Zampella et al. 2007, Andrén and Jarlman 2008, Cantonati and Lange-Bertalot 2011). In a study on epilithic diatoms in New Jersey Pinelands blackwater streams (pH 4.2 – 5.1), Zampella et al. (2007) recorded a mean species richness of 23.8, with *Eunotia exigua*, *Eunotia tenella* (Grunow) Hustedt and *Eunotia paludosa* Grunow as the most abundant taxa. While the naturally acidic streams were primarily dominated by acid-tolerant taxa, species from all pH classes were found (Zampella et al. 2007). Diversity in naturally acidic streams may be closely related to the concentration of dissolved organic carbon (DOC). Passy et al. (2006) observed high diatom diversity in a chronically acidified brown water stream with elevated concentrations of DOC and Al, primarily in the non-toxic organic form. In contrast, the episodically acidified clear water stream had much lower DOC levels and higher concentrations of toxic, inorganic Al. Diversity in this stream was low, and dominated by a single species, *E. exigua*.

Within New Zealand, studies on naturally acidic streams have typically focused on macroinvertebrates (Winterbourn and Collier 1987, Winterbourn and McDiffett 1996) or fish (Collier et al. 1990, Greig et al. 2010), but rarely algae (but see Collier and Winterbourn 1990). The presence of naturally acidic streams in close proximity to AMD streams has the potential to complicate the use of diatoms in AMD monitoring in New Zealand, as species typical of circum-neutral environments may have evolved to tolerate more acidic conditions. I am not aware of any research that compares the diatom communities in AMD and naturally acidic streams (but see Bray et al. 2008). West Coast streams provide an opportunity to do this.

1.5. Diatoms as environmental indicators

It has become standard practice to include both chemical and biological data in stream health assessment (e.g. USEPA National Water Quality Inventory 2007). Biota respond to cumulative environmental effects over time, which may be missed by spot water chemistry measurements of a limited number of parameters. Because a common objective of monitoring programs is to assess the potential impact of a stressor on aquatic biota, collecting data on the biota itself may provide the most valuable information (Dixit et al. 1992). Ideally, biological indicators should be easily measured, sensitive to stress and respond in a predictable manner to disturbance (Dale and Beyeler 2001). They should be widely distributed, both within the waterbody and across large geographical regions (Dixit et al. 1992). Fish, benthic macroinvertebrates and algae satisfy the above requirements and are commonly used aquatic bioindicators. While each has its own unique advantages and disadvantages (see Resh 2008), diatoms are increasingly recognised as important components of stream health assessment (Stevenson et al. 2010). In particular, diatoms have advantages in that very small samples are required for analysis and these often contain a diverse community; a single sediment sample may contain over 100 taxa (Dixit et al. 1992). For many diatom species, the environmental tolerances to parameters such as pH (van Dam et al. 1994) and conductivity (Potapova and Charles 2003) are known, and thus a shift in community composition can be used to indicate environmental change.

Diatoms respond rapidly to change, and act as early indicators of stream degradation and remediation (Stevenson et al. 2010).

Diatoms have been shown to respond predictably to a variety of anthropogenic stressors, and are incorporated into river monitoring studies in Europe (Kelly et al. 1998, Prygiel 2002), North America (Bahls 1993, Lavoie et al. 2009) and South America (Salomoni et al. 2006). In 2002, 20 states in the U.S. used periphyton and diatoms in routine bioassessment programs and five states had programs under development (USEPA 2002). In Europe, the EU Water Framework Directive incorporates the composition and abundance of diatoms into stream health assessment (European Parliament Directive 2000/60/EC). Approximately 20 different methods using benthic diatoms have been developed to assess European streams and rivers (Prygiel et al. 1999). Despite their wide use elsewhere, no diatom-based indices have yet been developed for New Zealand (C. Kilroy, NIWA, personal communication), and indices developed in Europe have not been tested on New Zealand streams (Biggs 2000). The New Zealand Periphyton Guidelines highlight the need for more research on algal indices (Biggs 2000). When algae are incorporated into New Zealand stream assessment, it is typically as a measure of biomass or cover (Biggs 2000). While these metrics may be adequate to assess nutrient enrichment, other stressors such as AMD may not have a significant relationship with biomass (Niyogi et al. 2002). Similarly, there have been few studies on algae in AMD-contaminated streams within New Zealand (Winterbourn et al. 2000, Novis 2006, Bray et al. 2008), and no previous studies with a focus on diatoms within these systems.

1.6. Thesis structure

The general aim of this thesis research was to identify diatom communities along a gradient of AMD on the West Coast, South Island, and determine if diatoms can be used to indicate the presence and magnitude of AMD within New Zealand streams. One significant challenge was the identification of diatoms to a species level. Although a number of guides and keys provided the ability to do this (e.g. Krammer and Lange-Bertalot 1991a, b, 1997, 2008), it was considered that a regional guide to common taxa would benefit water managers and regulators. Chapter 2 presents this illustrated guide to the most frequently observed diatom taxa in reference (both naturally acidic and circum-neutral) and AMD streams. Chapter 3 presents the results of a survey of diatom community composition and diversity along an AMD gradient and their relationship to environmental variables. Chapter 4 describes the development of two diatom-based indices for streams receiving AMD: a single Biotic Index and a Multimetric Index. These were compared to determine which best categorises streams into varying levels of AMD impact. Chapter 5 addresses the response time of diatoms to a rapid change in water chemistry. The focus is on the results of a translocation experiment in which natural substrate with mature algal biofilms was reciprocally transferred between circum-neutral reference, moderately, and severely impacted streams. Finally, Chapter 6 synthesises results and suggests areas of future research. Chapters 3 – 5 are written as draft scientific papers. Thus, there is some repetition, particularly in Introductions and Methods, between chapters.

Chapter 2

Illustrated guide to diatoms in streams on the West Coast, South Island, New Zealand

2.1. Introduction

Diatoms (Class: Bacillariophyceae) are a type of microscopic unicellular or colonial algae. They range in length from five to several hundred micrometres (Lowe 2011), and can be either benthic or planktonic. There are an estimated 200,000 extant species of diatoms, although only approximately 10,000 have been described, making them one of the most specious groups of algae (Mann and Droop 1996). Diatoms inhabit almost all aquatic habitats, from Antarctic lakes to geothermal hot springs (Jones 1996, Owen et al. 2008), and many species are greatly restricted in their habitat requirements (Stevenson et al. 2010). Their diversity, ubiquity across a variety of aquatic habitats, and autecology particular to each species make diatoms increasingly popular in biological monitoring to assess both past and present environmental change.

A basic understanding of morphology is necessary before diatoms can be identified. There is an extensive and detailed literature on diatom morphology (e.g. Barber and Haworth 1981, Round et al. 1990, Cox 2011) and this will be described only briefly here. The distinguishing feature of diatoms is their siliceous cell wall. The cell wall comprises two valves that fit together like the top and base of a Petri dish and are connected by girdle bands. The valves plus the girdle bands form a frustule, which is a protective covering for the internal living cell contents. Diatom valves form a variety of distinct shapes and are ornamented with several features that are taxonomically diagnostic (Fig. 2.1). It is often necessary to observe the specimen in valve (‘top’) view rather than girdle (‘side’) view for accurate identification, although in some genera viewing from both aspects is useful (Cox 2011). Two features that are commonly used in diatom identification are the density and orientation of striae as well as the shape and position of the raphe. Striae are visible as lines on the valve face. Striae may or may not be resolvable as rows of small pores (termed puncta or areolae) when using a light microscope. Puncta are simple pores, whereas areolae have internal or external substructures. The raphe is used for movement and is typically two slits that run along the midline of the valve (Round et al. 1990). Genera may be monoraphid (with one raphe valve and one araphid valve), biraphid (with a raphe present on both valves), or araphid (lacking raphes entirely). Araphid genera lack significant motility. Surrounding the raphe is an area devoid of striae termed the axial area. The axial area opens to the central area, which varies in size and shape across species. These as well as additional characteristic features allow diatoms to be identified to species or even variety.

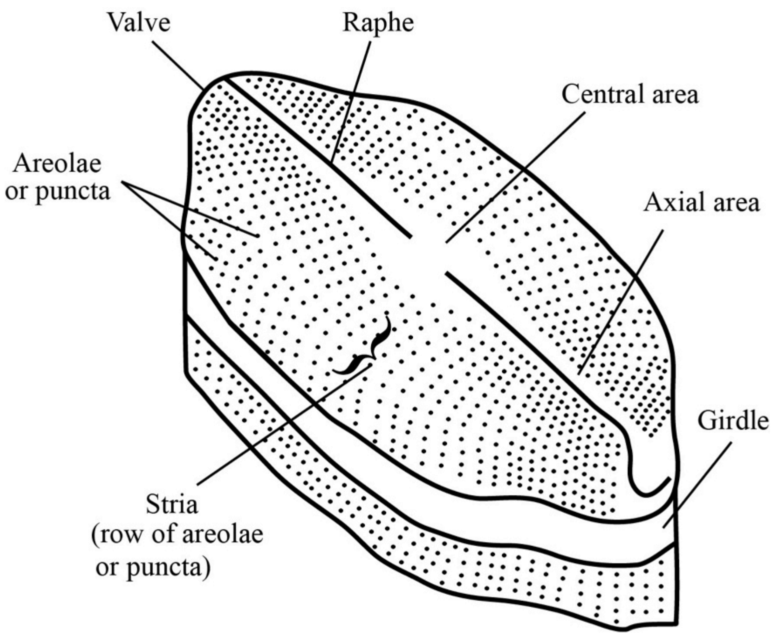


Figure 2.1. Three-dimensional representation of a diatom frustule with major diagnostic features labelled. Image adapted from Round et al. (1990, fig. 5B, p. 5).

One of the greatest challenges in incorporating diatoms into biological monitoring is accurate identification (Prygiel et al. 2002). The European diatom flora of Krammer and Lange-Bertalot (1991a, b, 1997, 2008) is arguably the most complete taxonomic guide and is used for species identification worldwide, including within New Zealand (e.g. Kilroy et al. 2006). These guides are extensive, containing information on over 500 *Navicula* species alone (Krammer and Lange-Bertalot 2008). Water managers and regulators may benefit from narrower, region-specific guides that focus on taxa likely to be found in an area of interest (Round 1991). To this aim, a photographic guide has been developed for common diatom taxa found on the West Coast of the South Island, New Zealand. A large number of streams within this region are impacted by acid mine drainage (AMD). Thus, the guide contains taxa found in naturally circum-neutral clear waters, naturally acidic brown waters, and AMD-contaminated streams. Several previous studies have shown that diatom communities of AMD streams are significantly different from reference streams and may be useful in identifying the presence and magnitude of AMD (Verb and Vis 2005, Smucker and Vis 2009, Zalack et al. 2010). Knowledge of the West Coast diatom flora characteristic of both stream types would be particularly useful if diatoms were to be incorporated into monitoring AMD impact in New Zealand. To my knowledge, this is the first diatom taxonomic guide written specifically for this region.

2.2. Methods

2.2.1. Diatom sample collection and slide preparation

Thirty-nine streams in the Buller and Grey Districts on the West Coast of the South Island, New Zealand were sampled on a single occasion between January and April 2011 (Appendix A). No standing waterbodies were sampled. Streams were assigned to one of four impact categories based on water chemistry (pH, specific conductivity, dissolved Al and Fe concentrations) and percentage iron hydroxide cover: circum-neutral reference (Ref. C) (pH > 5.7), naturally acidic reference (Ref. NA) (pH ≤ 5.7), moderate AMD, and severe AMD. See section 3.2.5 for further details regarding stream classification.

At each stream, a reach 10x the wetted width was established. Epiphytic diatoms were sampled at each stream by collecting visible filamentous algae and moss in all available habitats throughout the length of the study reach. Algae and moss were gently scraped from the substrate and combined with stream water into a 150 mL composite sample. Samples were preserved with Lugol's iodine and stored at 4º C until analysis. In the laboratory, samples were vigorously shaken and a 5 mL sub-sample of the algal suspension as well as a small clipping of plant material was placed in a 50 mL test tube. Approximately 10 mL of concentrated sulphuric acid and a few drops of 30% hydrogen peroxide were added to the sample to remove organic material (Biggs and Kilroy 2000). The sample was topped up with distilled water and allowed to settle. Samples were rinsed with distilled water 8 – 10 times to remove the oxidizing agents, with a minimum seven-hour settling period between rinses. A sub-sample of cleaned diatoms was transferred to a coverglass and allowed to air dry at room temperature. Permanent slides were created using the mounting medium Naphrax (Brunel Microscopes Ltd, Wiltshire, UK).

2.2.2. Diatom identification and enumeration

Slides were observed using a Zeiss AxioImager.M1 microscope (Carl Zeiss (NZ) Ltd, Auckland, New Zealand) equipped with a Differential Interference Contrast (DIC) system. Diatoms were viewed with a 100x EC Plan-NEOFLUAR objective with oil immersion and W-PI10x/43 eyepieces. Images were captured using a Zeiss AxioCam HRc CCD camera with AxioVision Rel. 4.5 software (2600 x 2060 scanned pixel resolution). Images were cropped, rotated, and 10 µm scale bars were added with Adobe Photoshop 7.0. No additional image adjustments were performed. Four hundred diatoms were identified per stream to the lowest taxonomic level possible (typically species) by transect scanning using the keys of Krammer and Lange-Bertalot (1991a, b, 1997, 2008), Patrick and Reimer (1966, 1975), Biggs and Kilroy (2000), Krammer (2000, 2002), Lange-Bertalot (2001), and Furey et al. (2011). For each species, 10 valves were measured to the nearest micrometre for traits such as length, width, and striae density using the program AxioVision Rel. 4.5. A note was made when fewer than 10 valves were measured due to the rarity of the species.

2.2.3. Diatom guide

The diatom guide includes all taxa present in > 3 streams, as well as a selection of rare taxa (those found in ≤ 3 streams) with distinct characteristics. The guide is organised alphabetically by family, followed by genus and species. Generic descriptions were summarised from Round et al. (1990), Patrick and Reimer (1966, 1975), Kingston (2003), and Kociolek and Spaulding (2003). For each species, the name and authority is provided as well as the main literature used for identification. There is then a brief description of valve morphology. Species descriptions consist of traits that are visible under a light microscope (LM); additional traits that would require a scanning electron microscope (SEM) are published elsewhere (e.g. Krammer 2000, 2002). Species descriptions are followed by an image of each diatom in valve (and occasionally girdle) view to aid in identification. A table is then provided with maximum and minimum values of each measured character in the present study as well as the range of measurements found in previous studies. When striae were un-resolvable in LM this is denoted as UR in the table. The literature used for measurement and identification may differ as this was based on the quality of the images and species description. The typical ecology of each species is then described, both in previous studies as well as in the present study. Species were described as being found in low (< 5%), moderate (5 – 20%) or high (> 20%) relative abundance in a stream. This is followed by a list of the stream number and impact category where the species was located, as well as its relative abundance (%, rounded to the nearest whole number) within each stream. For example, "Ref. C: 2 (15)" would indicate that a species was found in 15% relative abundance at Site #2, which is a circum-neutral reference stream. Species found in less than 1% relative abundance are recorded as < 1. For each species, streams with a relative abundance greater than 20% are indicated by bold typeface.

2.3. Illustrated diatom guide

Family: Achnanthidiaceae

Genus: *Achnanthidium* Kützing 1844

Achnanthidium is a monoraphid genus. Valves are lanceolate, linear or elliptic. In girdle view cells form a shallow V, with a concave raphid valve and convex araphid valve. Striae are typically uniseriate (striae composed of one row of areolae). Cells are usually small, either solitary or forming short chains and attached to the substrate by short stalks.

Achnanthidium minutissimum (Kützing) Czarnecki 1994
Krammer and Lange-Bertalot (1991b): pl. 32, figs 1 – 14, p. 312 as *Achnanthes minutissima* Kützing 1833

Description: Valves are narrowly elliptic with slightly rostrate apices (Fig. 2.2A). Striae are dense and may be difficult to make out in LM. In girdle view, the frustule forms a shallow V with slightly flattened ends (Fig. 2.2B). See Barber and Haworth (1981) for an illustrated guide to valve shapes and end types, as well as striae arrangement.

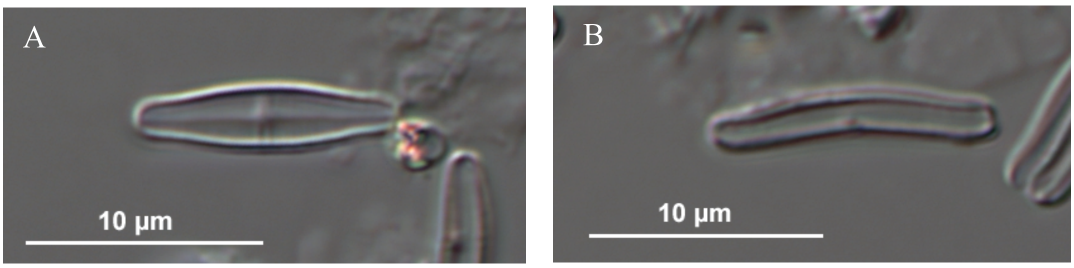


Figure 2.2. *A. minutissimum* valve view (A) and girdle view (B).

Taxon	Length (µm)	Width (µm)	Striae / 10 µm (araphid valve)	Striae / 10 µm (raphid valve)
**A. minutissimum*	10 – 16	2 – 3	30	30
***Achnanthes minutissima* - Patrick and Reimer (1966)	5 – 40	2 – 4	30 – 32	30 – 32

*The first row of measurements is from the present study. This applies to all tables below.
**The second row of measurements is from the literature listed after the species name in the table. This applies to all tables below.

Ecology: This species is common in New Zealand and found across a variety of water chemistries (Biggs and Kilroy 2000), although it typically inhabits circum-neutral streams (van Dam et al. 1994). It is often the first species to colonise a site after a scouring event and therefore may dominate streams in regions with high annual rainfall, such as New Zealand's West Coast (Stevenson and Bahls 1999). In the present study, *A. minutissimum* was primarily found in circum-neutral reference streams in low to high relative abundance.

Site(s) (% relative abundance):
Ref. C: 1 (13), 5 (< 1), 9 (< 1), 12 (2), 13 (4), 14 (< 1), **22** (36), 23 (2), 24 (< 1), 37 (1), 39 (1)
Ref. NA: 26 (<1)
Moderate: 4 (1), 11 (< 1), 15 (7), 16 (2)
Severe: 34 (< 1)

Genus: *Karayevia* Round & L. Bukhtiyarova ex. F.E. Round 1998

Karayevia is a monoraphid genus. Valves are linear to lanceolate with rounded, rostrate, or capitate apices. Striae radiate on the raphid valve, and are parallel on the araphid valve. Distal raphe fissures curve in the same direction. Cells of *Karayevia* are typically found prostrate on sand grains (epipsammic).

Karayevia oblongella (Østrup) M. Aboal 2003
Krammer and Lange-Bertalot (1991b): pl. 16, figs 1 – 14, p. 280 as *Achnanthes oblongella* Østrup 1902

Description: Valves are elliptic with broadly rounded apices. Striae are coarse on the araphid valve and may appear bent and irregular in length (Fig. 2.3A). Striae are parallel in the centre of the valve and radiate at the poles. Striae of the raphid valve are fine and radiate from a bowtie-shaped central area (Fig. 2.3B).

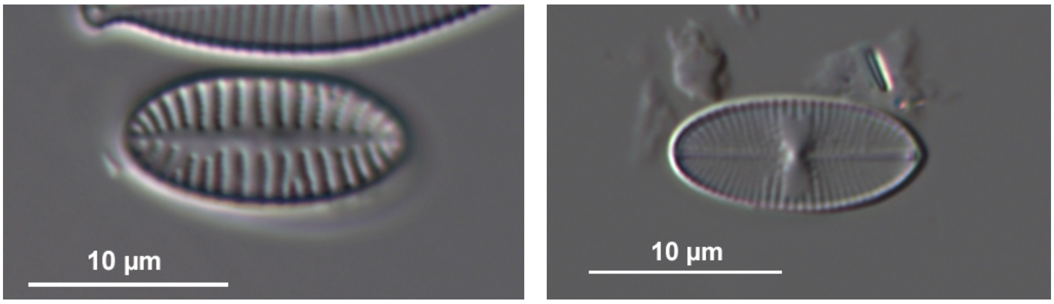


Figure 2.3. *K. oblongella* araphid valve (A) and raphid valve (B).

Taxon	Length (µm)	Width (µm)	Striae / 10 µm (araphid valve)	Striae / 10 µm (raphid valve)
K. oblongella	13 – 16	6 –7	11 – 12	23 – 30
Achnanthes oblongella - Patrick and Reimer (1966)	10 – 20	4 – 8	9 – 12	24 – 30

Ecology: This species has been reported as sensitive to metal pollution (Hirst et al. 2002). It mainly occurs in circum-neutral streams and is widespread throughout New Zealand (van Dam et al. 1994, Biggs and Kilroy 2000). In the present study, *K. oblongella* was found primarily in circum-neutral reference streams in low to high relative abundance.

Site(s) (% relative abundance):
Ref. C: 1 (< 1), 2 (3), 5 (19), 8 (1), 9 (18), 12 (4), 13 (1), 14 (7), 22 (< 1), 23 (19), **24** (53), 25 (8), **31** (40), 37 (4), 38 (4), **39** (55)
Ref. NA: 3 (2), 26 (7)
Moderate: 4 (9), 6 (3), 7 (< 1), 11 (6), 15 (15), 16 (< 1), 36 (< 1)
Severe: 19 (< 1), 32 (< 1)

Genus: *Planothidium* Round & L. Bukhtiyarova 1996

Planothidium is a monoraphid genus. Valves are elliptic to lanceolate with rounded, capitate or rostrate apices. Striae are multiseriate (striae composed of more than one row of areolae) and radiate on both valves. There is either a horseshoe-shaped depression or hood in the central area of the araphid valve on most species.

Planothidium haynaldii (Schaarschmidt) Lange-Bertalot 1999
Krammer and Lange-Bertalot (1991b): pl. 41, figs 16 – 20, p. 330 as *Achnanthes lanceolata* ssp. *lanceolata* var. *haynaldii* (Schaarschmidt) Cleve 1894

Description: Valves are lanceolate to elliptic with capitate apices. Striae of both the raphid and araphid valve are radiate throughout. A horseshoe-shaped depression is present on one side in the centre of the araphid valve (Fig. 2.4A). Axial area is narrow and linear. Central area of the raphid valve is elliptical to bowtie-shaped (Fig. 2.4B).

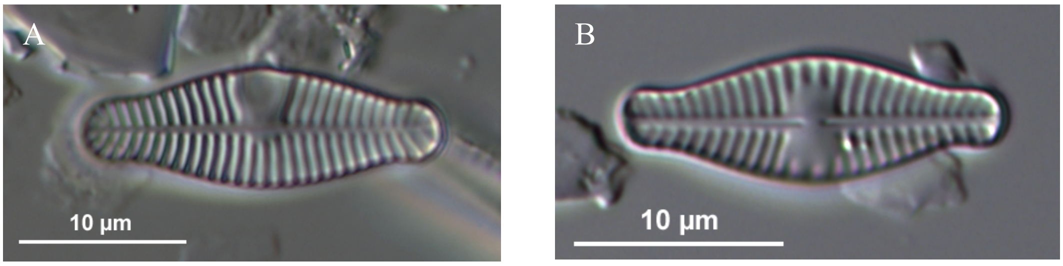


Figure 2.4. *P. haynaldii* araphid valve (A) and raphid valve (B). Note the capitate apices.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm (araphid valve)	Striae / 10 µm (raphid valve)
P. haynaldii	16 – 22	6 – 7	13 – 14	13 – 14
Achnanthes lanceolata ssp. *lanceolata* var. *haynaldii* - Krammer and Lange-Bertalot (1991b)	6 – 40	4.5 – 10	10 –15	10 – 15

Ecology: This species prefers a slightly alkaline environment (van Dam et al. 1994). It is often found with *Planothidium lanceolatum* (Patrick and Reimer 1966). In the present study, *P. haynaldii* was found in both circum-neutral reference and moderately impacted streams.

Site(s) (% relative abundance):
Ref. C: 13 (5), 37 (< 1), 38 (1)
Moderate: 7 (< 1)

Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot 1999
Krammer and Lange-Bertalot (1991b): pl. 41, figs 1 – 8, p. 330 as *Achnanthes lanceolata* (Brébisson ex Kützing) Grunow 1880

Description: Valves are lanceolate to elliptic with rounded apices. Striae radiate on both valves. Two or more central striae of the raphid valve are shortened forming an elliptical to slightly bowtie-shaped central area (Fig. 2.5A). A horseshoe-shaped depression is present on one side of the central area of the araphid valve (Fig. 2.5B). Axial area is narrow and linear on both valves.

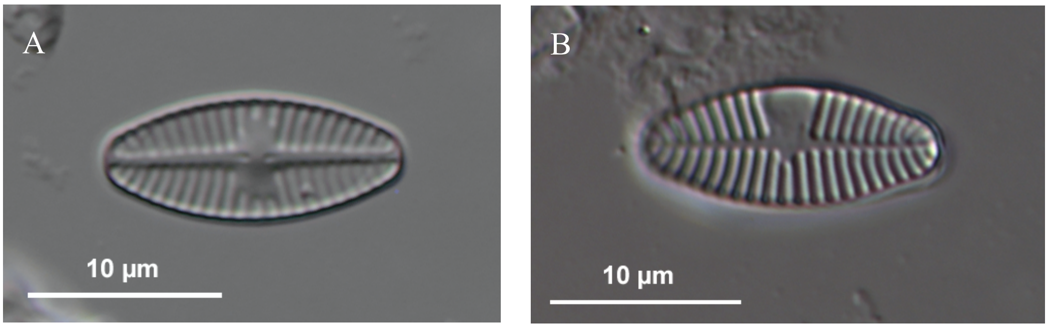


Figure 2.5. *P. lanceolatum* raphid valve (A) and araphid valve (B).

Taxon	Length (µm)	Width (µm)	Striae / 10 µm (araphid valve)	Striae / 10 µm (raphid valve)
P. lanceolatum	12 – 22	5 – 8	12 – 15	11 – 14
Achnanthes lanceolata - Patrick and Reimer (1966)	12 – 31	4.5 – 8	11 – 14	11 – 14

Ecology: This species is common in New Zealand and can be found in a variety of water chemistries (Biggs and Kilroy 2000). It typically prefers oxygen-rich waters of circum-neutral to slightly alkaline pH (Patrick and Reimer 1966). In the present study, *P. lanceolatum* was found in greatest relative abundance in circum-neutral reference streams. It was also found in streams moderately impacted by AMD, but in low relative abundance.

Site(s) (% relative abundance):
Ref. C: 1 (4), 2 (5), **5** (63), **8** (23), 9 (3), 12 (6), 13 (5), 14 (< 1), 23 (< 1), 24 (1)
Moderate: 4 (< 1), 6 (< 1), 7 (< 1), 16 (2)

Genus: *Rossithidium* Round & L. Bukhtiyarova 1996

Rossithidium is a monoraphid genus. Valves are linear to elliptic with rounded apices. Striae are parallel or slightly radiate throughout the valve and are often fine and closely spaced. The raphe is straight and lies in a narrow axial area.

Rossithidium lineare (W. Smith) Round & L. Bukhtiyarova 1996
Patrick and Reimer (1966): pl. 16, figs 3 – 4, p. 286 as *Achnanthes linearis* (W. Smith) Grunow 1880

Description: Valves are narrowly elliptic with rounded apices. Striae of both the raphid and araphid valve are parallel and closely and evenly spaced, becoming radiate at the poles (Fig. 2.6A, B). Axial area is narrow and linear.

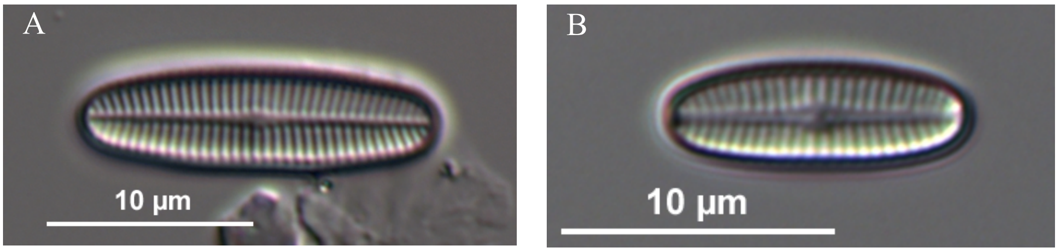


Figure 2.6. *R. lineare* araphid valve (A) and raphid valve (B).

Taxon	Length (μm)	Width (μm)	Striae / 10 μm (araphid valve)	Striae / 10 μm (raphid valve)
R. lineare	10 – 21	3 – 4	22 – 24	21 –2 6
Achnanthes linearis - Patrick and Reimer (1966)	10 – 20	2.5 – 3.5	23 – 26	23 – 26

Ecology: This species can tolerate a range of water chemistries (Biggs and Kilroy 2000); although it is typically found in circum-neutral streams (van Dam et al. 1994). In the present study *R. lineare* was primarily found in circum-neutral reference streams in low to high relative abundance.

Site(s) (% relative abundance):

Ref. C: 1 (16), 2 (8), 5 (1), 8 (1), 9 (4), 12 (16), 13 (16), 14 (13), 22 (5), 24 (7), 25 (< 1), 31 (< 1), 37 (4), 38 (8), **39** (28)

Moderate: 7 (< 1), 15 (< 1), 16 (4)

Family: Amphipleuraceae

Genus: *Frustulia* Rabenhorst 1853

Valves are lanceolate, linear, or rhombic with rounded to capitate apices. Margins are straight to undulate. Raphe lies on a thickened rib that extends across the length of the valve. Cells are either solitary or colonial in mucilaginous tubes.

Frustulia crassinervia (Brébisson) Lange-Bertalot & Krammer 1996
Patrick and Reimer (1966): pl. 22, fig. 1, p. 342 as *Frustulia rhomboides* var. *crassinervia* (Brébisson) Ross 1947

Description: Valves are elliptic to lanceolate with rostrate apices. Valve margin is slightly undulate in appearance. Striae are fine and difficult to distinguish in LM. Central area is rectangular in shape (Fig. 2.7).

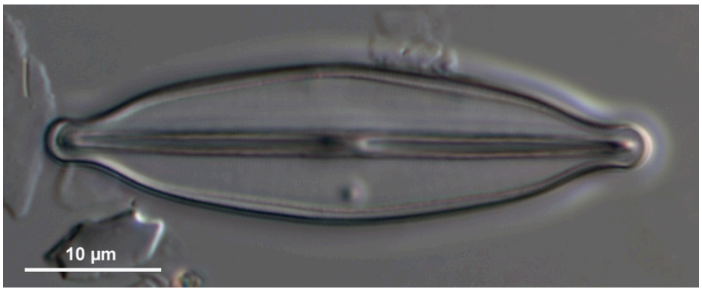


Figure 2.7. *F. crassinervia* valve. Note the undulating valve margins.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
F. crassinervia	35 – 46	10 – 11	UR
F. rhomboides var. *crassinervia* - Patrick and Reimer (1966)	30 – 50	10 – 15	40

Ecology: This species is typically found in streams of pH < 5.5 that are buffered by humic acids (van Dam et al. 1994, Lange-Bertalot 2001). In the present study, *F. crassinerva* was found in circum-neutral, naturally acidic, and moderately impacted streams, with highest relative abundance in a naturally acidic stream.

Site(s) (% relative abundance):
Ref. C: 5 (< 1), 9 (< 1), 24 (< 1), 38 (1), 39 (2)

Ref. NA: 3 (7), 10 (11), **21** (21), 26 (< 1)

Moderate: 6 (1), 7 (2), 11 (1), 20 (7)

Frustulia rhomboides (Ehrenberg) De Toni 1891
Patrick and Reimer (1966): pl. 21, fig. 5 p. 340
Krammer and Lange-Bertalot (2008): pl. 95, figs 1 – 3, p. 630

Description: Valves are rhombic with broadly rounded apices. Striae are fine and may be difficult to resolve in LM. Central area is rectangular in shape, slightly pinched in the centre (Fig. 2.8).

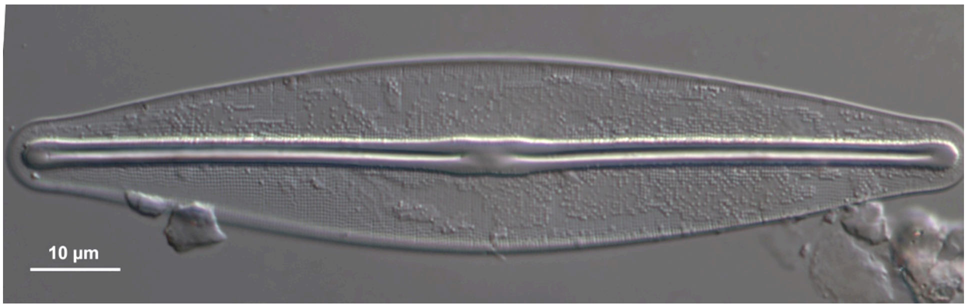


Figure 2.8. *F. rhomboides* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
F. rhomboides	72 – 94	17 – 21	26 – 30
F. rhomboides - Patrick and Reimer (1966)	70 – 160	15 – 30	20 – 30

Ecology: This species is widespread throughout New Zealand and may be abundant (Biggs and Kilroy 2000). It prefers streams of pH < 7 and is indicative of acidic environments (van Dam et al. 1994, DeNicola 2000). In a U.S. study, Verb and Vis (2000) found this species dominated a stream receiving AMD of pH 2.6 – 3.5. In the present study, *F. rhomboides* was found in a variety of water chemistries, although it was in highest relative abundance in naturally acidic and moderately impacted streams.

Site(s) (% relative abundance):

Ref. C: 8 (< 1), 14 (2), 25 (< 1), 37 (2)

Ref. NA: 10 (2), 21 (5)

Moderate: 15 (2), 20 (5)

Severe: 35 (< 1)

Frustulia rhomboides var. *capitata* (Mayer) R.M. Patrick 1966
Patrick and Reimer (1966): pl. 21, fig. 8, p. 340.

Description: Valves are linear to slightly lanceolate with capitate apices. Striae are fine and difficult to make out under LM. Central area is rectangular in shape, pinched in the centre (Fig. 2.9).

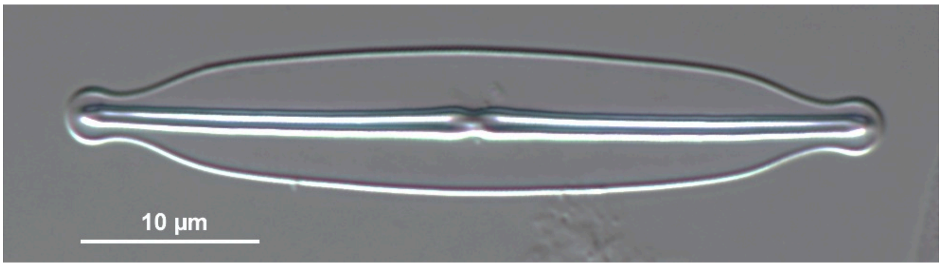


Figure 2.9. *F. rhomboides* var. *capitata* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
F. rhomboides var. *capitata*	46 – 57	9 – 13	UR
F. rhomboides var. *capitata* - Patrick and Reimer (1966)	40 – 60	10 – 13	24 – 30

Ecology: This species prefers slightly acidic environments (Patrick and Reimer 1966). In the present study, *F. rhomboides* var. *capitata* was found in low to moderate relative abundance in naturally acidic streams and low relative abundance in moderately impacted streams.

Site(s) (% relative abundance):

Ref. NA: 3 (8), 10 (3), 21 (14)

Moderate: 6 (1), 7 (2), 11 (< 1), 15 (< 1), 16 (1), 20 (3)

Frustulia saxonica Rabenhorst 1853
Krammer and Lange-Bertalot (2008): pl. 95, figs 4 – 5, p. 630 as *Frustulia rhomboides* var. *saxonica* (Rabenhorst) De Toni 1891

Description: Valves are rhombic to lanceolate with broadly rounded apices. Striae are fine and parallel in the centre of the valve, slightly radiate towards the poles. Central area is rectangular in shape, slightly pinched in the centre (Fig. 2.10).

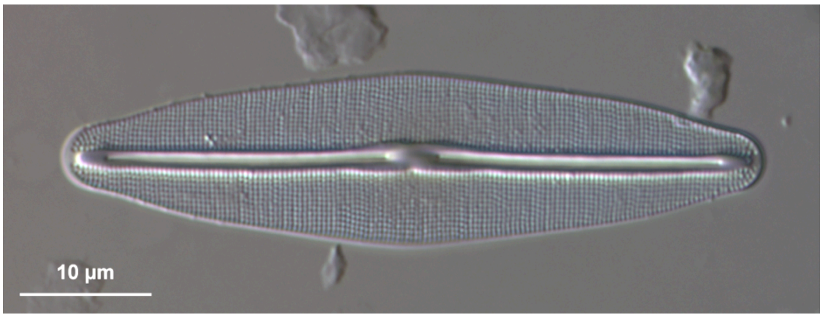


Figure 2.10. *F. saxonica* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
F. saxonica	40 – 65	10 – 13	UR
F. rhomboides var. *saxonica* - Patrick and Reimer (1966)	40 – 70	12 – 20	36 – 40

Ecology: This species is typically found in acidic environments that are buffered with humic acids (van Dam et al. 1981, 1994, Lange-Bertalot 2001). *F. saxonica* has been reported in AMD streams within the U.S., although it is not common in streams of pH < 3.5 (DeNicola 2000 and references within). In the present study, *F. saxonica* was found in greatest relative abundance in a naturally acidic reference stream.

Site(s) (% relative abundance):
Ref. C: 9 (1), 24 (6), 25 (4), 37 (1), 38 (2), 39 (4)
Ref. NA: 3 (< 1), **10** (21), 21 (13), 26 (1)
Moderate: 6 (1), 7 (3), 11 (2), 15 (10), 16 (< 1), 20 (4)

Frustulia vulgaris (Thwaites) De Toni 1891
Krammer and Lange-Bertalot (2008): pl. 97, figs 1 – 6, p. 634
Patrick and Reimer (1966): pl. 22, fig. 3, p. 342

Description: Valves are elliptic to lanceolate with rounded apices. Raphe may appear slightly curved. Axial area is narrow, opening to an elliptical central area that is longer than it is wide. Striae are fine and may be difficult to make out in LM (Fig. 2.11).

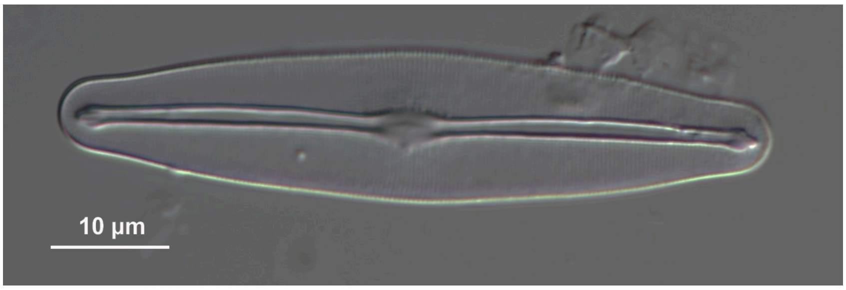


Figure 2.11. *F. vulgaris* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
F. vulgaris	50 – 61	9 – 12	UR
F. vulgaris - Lange-Bertalot (2001)	40 – 60	8 – 12	27 – 32

Ecology: This species has a wide environmental tolerance, from freshwater to slightly brackish habitats (Lange-Bertalot 2001). It prefers streams of pH > 7 (van Dam et al. 1994). In the present study, *F. vulgaris* was found primarily in circum-neutral reference and moderately impacted streams in low relative abundance.

Site(s) (% relative abundance):

Ref. C: 8 (< 1), 9 (2), 13 (< 1), 22 (< 1), 24 (2), 37 (< 1), 38 (< 1), 39 (< 1)

Ref. NA: 10 (< 1)

Moderate: 4 (< 1), 11 (2), 15 (< 1), 20 (< 1), 36 (< 1)

Family: Bacillariaceae

Genus: *Nitzschia* Hassall 1845

Valves are typically linear to lanceolate, occasionally sigmoid. Striae are often fine or difficult to resolve under LM. The raphe typically runs along the valve margin but may also be positioned slightly eccentrically. The raphe itself is often not visible under LM. Fibulae, which may appear as small dots or box-like markings, are used to determine the location of the raphe. Species typically possess nitzschiod symmetry, in which the raphe system lies on opposite margins on each valve.

Nitzschia cf. *amabilis* Suzuki 2010

Krammer and Lange-Bertalot (1997): pl. 65, figs 1 – 2A, p. 346 as *Nitzschia laevis* Hustedt 1939

Description: Valves are elliptic with acutely rounded apices. Fibulae are small and irregularly spaced. A gap is present between the central pair of fibulae (Fig. 2.12).

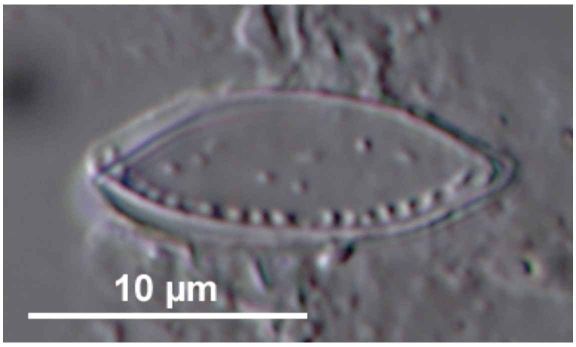


Figure 2.12. *N*. cf. *amabilis* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm	Fibulae / 10 µm
**N*. cf. *amabilis*	14 –17	5	UR	11 – 12
N. laevis - Krammer and Lange-Bertalot (1997)	(5) 12 – 26.5	(2.5) 4.4 – 7	32 – 36	10 – 14

*Fewer than 10 valves were measured.

Ecology: *Nitzschia amabilis* is primarily a marine species (Suzuki et al. 2010). In the present study, *N*. cf. *amabilis* was found in low relative abundance in streams moderately to severely impacted by AMD.

Site number (% relative abundance):

Moderate: 16 (< 1)

Severe: 19 (< 1), 28 (< 1), 34 (< 1)

Nitzschia dissipata (Kützing) Grunow 1862
Krammer and Lange-Bertalot (1997): pl. 11, figs 1 – 7, p. 238

Description: Valves are linear to lanceolate with rostrate apices. Fibulae are rectangular and irregular in width. Raphe is positioned eccentrically. Striae are difficult to resolve in LM (Fig. 2.13).

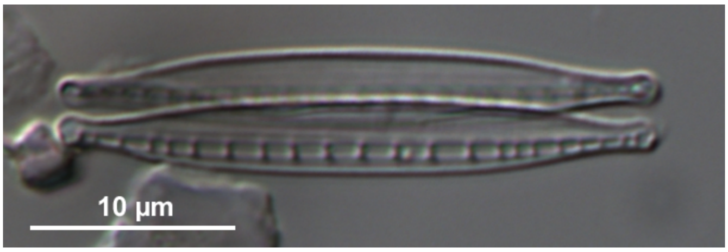


Figure 2.13. Two valves of *N. dissipata.* Note the slightly eccentric raphe.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm	Fibulae / 10 µm
N. dissipata	28 – 50	3 – 4	UR	7 – 10
N. dissipata - Krammer and Lange-Bertalot (1997)	12 – 85	3 – 7	32	5 – 11

Ecology: This species typically inhabits high-conductivity environments of pH > 7 (van dam et al. 1994, Biggs and Kilroy 2000). In the present study, *N. dissipata* was found predominately in circum-neutral reference streams in low relative abundance.

Site(s) (% relative abundance):

Ref. C: 1 (< 1), 2 (3), 8 (4), 12 (< 1), 13 (< 1), 22 (< 1)

Moderate: 15 (< 1)

Nitzschia palea (Kützing) W. Smith 1856
Krammer and Lange-Bertalot (1997): pl. 59, figs 1 – 24, p. 334

Description: Valves are linear to lanceolate with rostrate apices. Striae are dense and difficult to resolve in LM. Fibulae are small and dot-like and may appear irregularly spaced (Fig. 2.14).

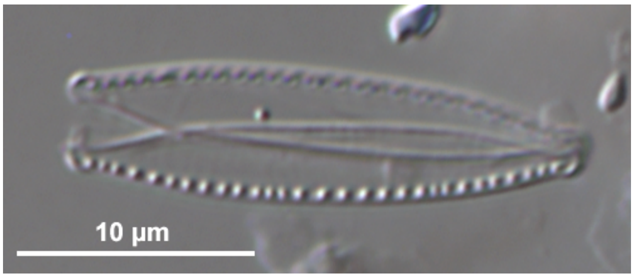


Figure 2.14. Two valves of *N. palea.*

Taxon	Length (μm)	Width (μm)	Striae / 10 μm	Fibulae / 10 μm
N. palea	22 – 36	3 – 4	UR	10 – 14
N. palea - Krammer and Lange-Bertalot (1997)	15 – 70	2.5 – 5	28 – 40	9 – 17

Ecology: This species is widespread and common in New Zealand (Biggs and Kilroy 2000). In the present study, *N. palea* was found in both circum-neutral reference and moderately impacted streams.

Site(s) (% relative abundance):
Ref. C: 1 (< 1), 2 (3), 22 (< 1), 24 (< 1)
Moderate: 6 (< 1), 11 (1)

Nitzschia paleaeformis Hustedt 1950
Krammer and Lange-Bertalot (1997): pl. 65, figs 3 – 8A, p. 346

Description: Valves are linear to lanceolate with rostrate apices. Striae are fine and difficult to make out in LM. Fibulae are small and dot-like, with a gap between the central pair of fibulae (Fig. 2.15).

Figure 2.15. *N. paleaeformis* valve. Note the gap between the central fibulae.

Taxon	Length (μm)	Width (μm)	Striae / 10 μm	Fibulae / 10 μm
N. paleaeformis	30 – 37	4 – 5	UR	10 – 12
N. paleaeformis - Krammer and Lange-Bertalot (1997)	30 – 90	3 – 5	35 – 40	10 – 13

Ecology: This species prefers streams of pH < 5.5 (van Dam et al. 1994). It has been recorded in lakes receiving AMD in Germany (Kapfer 1998) and Australia (John 1993). In the present study, *N. paleaeformis* was found exclusively in streams of pH ≤ 4.6.

Site(s) (% relative abundance):
Ref. NA: 10 (< 1)
Moderate: 11 (7), 16 (< 1)
Severe: **17** (31), 18 (8), 19 (6), 27 (15), 29 (< 1), 33 (< 1)

Family: Brachysiraceae

Genus: *Brachysira* Kützing 1836

Valves are linear, lanceolate or rhombic with rounded apices. Striae are uniseriate and often visibly punctate. Axial area is narrow and linear. *Brachysira* cells grow singly, either unattached or stalked. Commonly epipelic.

Brachysira brebissonii R. Ross 1986

Patrick and Reimer (1966): pl. 33, figs 7 – 8, p. 426 as *Anomoeoneis serians* var. *brachysira* (Brébisson ex Rabenhorst) Hustedt 1930

Description: Valves are rhombic to lanceolate with rounded apices. Axial area is narrow, opening to a circular central area. Striae radiate throughout the valve and are visibly punctate (Fig. 2.16).

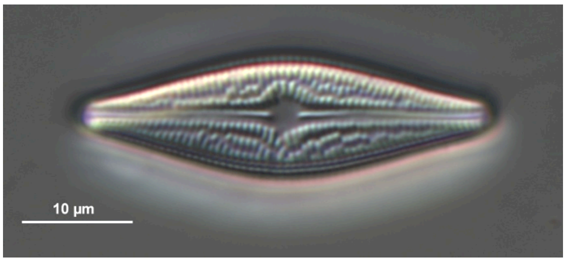


Figure 2.16. *B. brebissonii* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
B. brebissonii	16 – 25	6 – 7	24 – 26
Anomoeoneis serians var. *brachysira* - Patrick and Reimer (1966)	12 – 50	4 – 10	24

Ecology: This species is typically found in low conductivity streams of pH < 7.0 (van Dam et al 1994, Potapova and Charles 2003). It was found in high abundance in Australian lakes formed from coal mining of pH < 4.5 and conductivity 385 – 1931 µS/cm (Thomas and John 2006). In the present study *B. brebissonii* was primarily found in moderately impacted and naturally acidic streams.

Site(s) (% relative abundance):

Ref. C: 28 (< 1)

Ref. NA: 3 (< 1), 10 (2), 21 (3)

Moderate: 24 (3), 11 (1), 15 (7), 20 (4)

Family: Cocconeidaceae

Genus: *Cocconeis* Ehrenberg 1837

Cocconeis is a monoraphid genus. Valves are elliptic to nearly circular. Raphe is linear and central. Striae typically radiate on both valves. Cells are solitary and attached to plants or rocks by mucilage excreted from the raphid valve.

Cocconeis placentula Ehrenberg 1838
Krammer and Lange-Bertalot (1991b): pl. 51, figs 1 – 5, p. 350

Description: Valves are elliptic with rounded apices. The raphid valve has a narrow axial area and a small circular central area (Fig. 2.17A). Striae are slightly radiate and punctate. Two hyaline areas are present near the valve margin, interrupting the striae. The araphid valve has a narrow axial area with no visible central area (Fig. 2.17B). Striae of the araphid valve are slightly radiate and noticeably punctate. This species is rarely seen in girdle view.

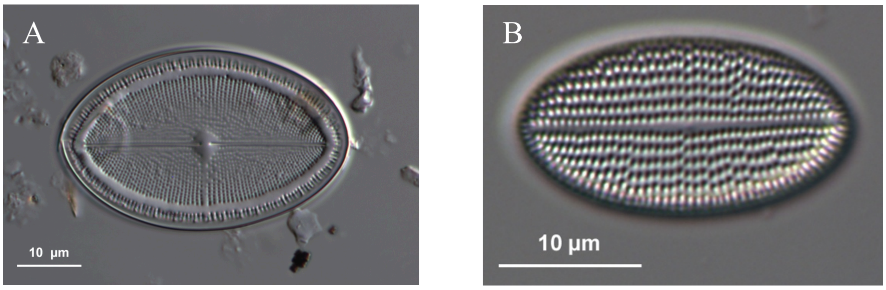


Figure 2.17. *C. placentula* raphid valve (A) and araphid valve (B).

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
C. placentula	21 – 25	10 – 13	18 – 22
C. placentula - Patrick and Reimer (1966)	10 – 70	8 – 40	20 – 23

Ecology: *Cocconeis placentula* is common and widespread throughout New Zealand (Biggs and Kilroy 2000). It may be found in a variety of conditions, from clean to enriched streams (Biggs and Kilroy 2000) and is typically epiphytic (Patrick and Reimer 1966). In the present study, *C. placentula* was found primarily in circum-neutral reference streams in low to high relative abundance.

Site(s) (% relative abundance):

Ref. C: 1 (< 1), 2 (< 1), 8 (< 1), 12 (5), 13 (2), **14** (32), 25 (2), **31** (53), **37** (47), 38 (< 1), 39 (< 1)

Ref. NA: 21 (< 1)

Moderate: 6 (< 1), 7 (< 1), 15 (2), **16** (26), 20 (< 1), 36 (< 1)

Severe: 34 (< 1)

Family: Cymbellaceae

Genus: *Cymbella* C. Agardh 1830

Valves are dorsiventral, semicircular to nearly lanceolate in shape with rounded, rostrate or capitate apices. One or more stigmata are typically present on the ventral side near the centre of the valve. Distal raphe fissures are deflected towards the dorsal margin, and proximal raphe fissures towards the ventral margin. Cells may be solitary, or colonial and stalked.

Cymbella aspera (Ehrenberg) Cleve 1894
Krammer and Lange-Bertalot (2008): pl. 131, figs 1 – 3, p. 704

Description: Valves are crescentic with broadly rounded apices. Dorsal margin is smoothly arched, ventral margin is straight to slightly gibbous in the centre of the valve. Axial area is fairly wide, opening to a central area that is longer than it is wide. Striae are radiate and noticeably punctate (Fig. 2.18). Seven to ten stigmata are present on the ventral side of the central area, but may be difficult to resolve in LM.

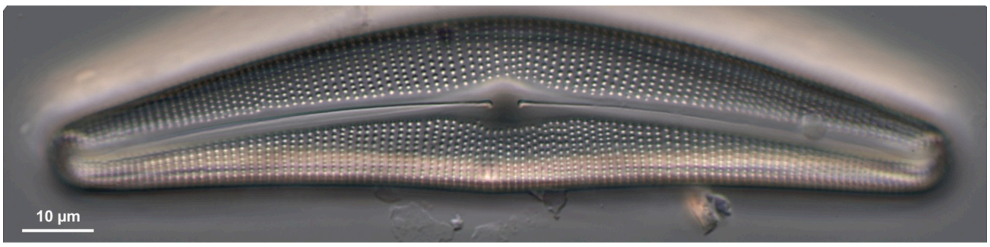


Figure 2.18. *C. aspera* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
**C. aspera*	80 – 145	20 – 25	8 – 9
C. aspera - Patrick and Reimer (1975)	70 – 200	20 – 30	7 – 10

*Fewer than 10 valves were measured.

Ecology: This species is typically found in mountain streams of moderate conductivity (Krammer 2002). It is often rare but has been shown to dominate some streams within North America and New Zealand (Krammer 2002). In the present study, *C. aspera* was found in low relative abundance in a circum-neutral reference and moderately impacted stream.

Site(s) (% relative abundance):

Ref. C: 23 (< 1)

Moderate: 4 (< 1)

Cymbella kappii (Cholnoky) Cholnoky 1956

Krammer (2002): pl. 51, figs 13 – 17, p. 292

Description: Valves are semicircular with a smooth dorsal margin and a slightly convex ventral margin. Ventral margin may be slightly gibbous in the centre. Apices are rounded to rostrate. Striae are parallel in the centre of the valve and radiate towards the poles. Two stigmata are present on the ventral side of the valve near the proximal raphe endings (Fig. 2.19).

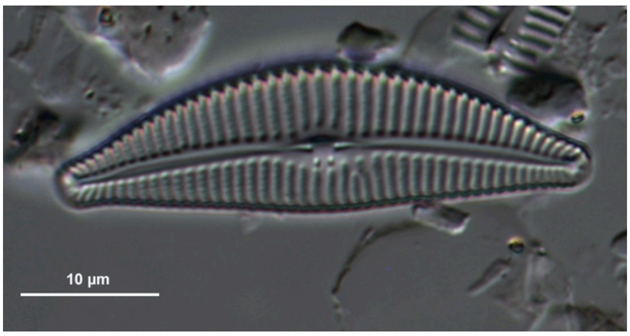


Figure 2.19. *C. kappii* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
C. kappii	30 – 40	10 – 12	9 – 11
C. kappii - Krammer (2002)	22 – 58	7 – 11	8 – 12

Ecology: This species is very common in New Zealand and is characteristic of un-polluted streams (Biggs and Kilroy 2000). It prefers streams of low to moderate conductivity (Krammer 2002). In the present study, *C. kappii* was found in low to high relative abundance in circum-neutral reference streams, as well as in a single moderately impacted stream in low relative abundance.

Site(s) (% relative abundance):
Ref. C: 1 (< 1), **2** (34), **12** (24), 13 (2), 14 (< 1)
Moderate: 16 (< 1)

Cymbella tumida (Brébisson) van Heurck 1880
Krammer (2002): pl. 162, figs 1 – 8, p. 514

Description: Valves are semicircular with a smooth, convex dorsal margin and a straight to slightly gibbous ventral margin. Apices are rostrate. Axial area is narrow, opening to a circular central area. A single stigma is present on the ventral side of the central area. Striae radiate near the centre and become parallel or convergent towards the poles (Fig. 2.20).

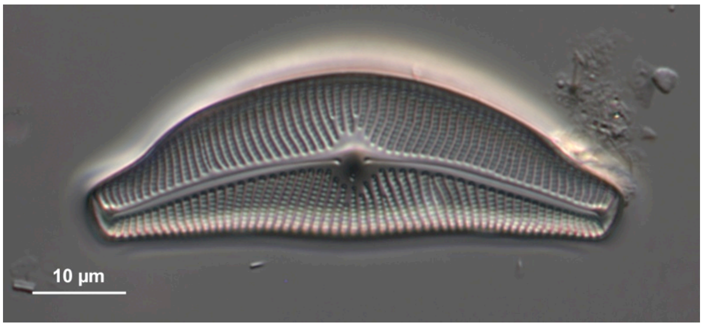


Figure 2.20. *C. tumida* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
C. tumida	54 – 65	16 – 19	9 – 10
C. tumida - Patrick and Reimer (1975)	25 – 80	12 – 18	8 – 10

Ecology: This species typically prefers alkaline streams of moderate conductivity and is common in samples from the North Island of New Zealand (Patrick and Reimer 1975, Biggs and Kilroy 2000, Krammer 2002). In the present study, *C. tumida* was found in low relative abundance in a single circum-neutral stream.

Site(s) (% relative abundance):
Ref. C: 31 (< 1)

Genus: *Encyonema* Kützing 1834

Valves are semicircular with rounded to capitate apices. Dorsal margin is arched and ventral margin is straight to slightly gibbous. Proximal raphe ends are deflected dorsally and distal raphe ends ventrally, which distinguishes this genus from *Cymbella*. *Encyonema* cells are either solitary or exist as colonies in mucilaginous tubes.

Encyonema minutum (Hilse) D.G. Mann 1990
Krammer and Lange-Bertalot (2008): pl. 119, figs 1 – 13, p. 680 as *Cymbella minuta* Hilse 1862

Description: Valves are semicircular with a straight ventral margin and a strongly arched dorsal margin. Apices are rounded and may bend ventrally. Central area is either small and rounded, or absent. Striae are parallel or slightly radiate (Fig. 2.21).

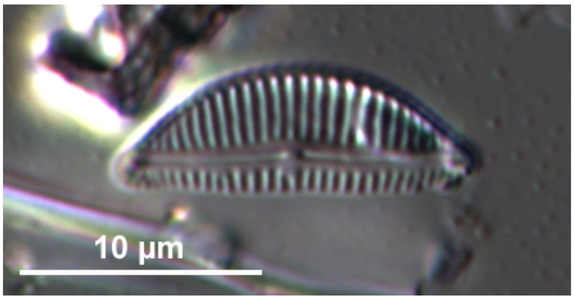


Figure 2.21. *E. minutum* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
E. minutum	12 – 19	5 – 6	14 – 17
Cymbella minuta - Patrick and Reimer (1975)	9 – 28	4.5 – 6	14 – 16

Ecology: This species is common in New Zealand, especially in developed catchments (Biggs and Kilroy 2000). It can tolerate a wide range of pH (Patrick and Reimer 1975). In the present study, *E. minutum* was primarily found in circum-neutral reference streams, where it was present in low to high relative abundance.

Site number (% relative abundance):

Ref. C: 1 (2), 2 (< 1), 9 (4), 12 (5), **13** (24), 22 (< 1), 23 (1), 24 (1), 25 (18), 38 (20), 39 (< 1)

Ref. NA: 26 (19)

Moderate: 7 (< 1), 11 (< 1), 15 (9), 16 (1)

Encyonema prostratum (Berkeley) Kützing 1844

Krammer and Lange-Bertalot (2008): pl. 123, figs 7 – 10, p. 688 as *Cymbella prostrata* (Berkeley) Cleve 1894

Description: Valves are semicircular with a curved dorsal margin and a slightly convex ventral margin. Apices are broadly rounded and bent ventrally. Linear axial area expands to a circular central area. Striae are radiate throughout and are present around the valve poles. Distal raphe ends are curved strongly towards the ventral margin (Fig. 2.22).

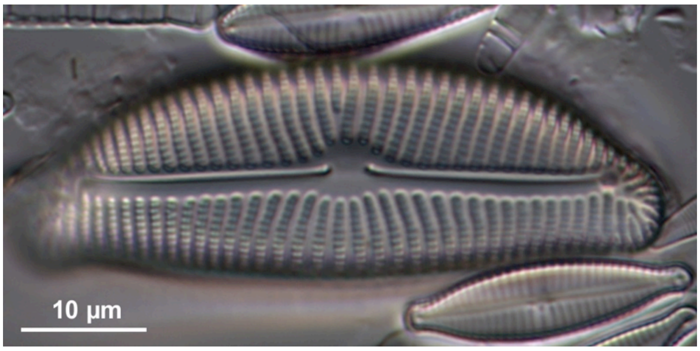


Figure 2.22. *E. prostratum* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
E. prostratum	47 – 58	17 – 21	7 – 9
Cymbella prostrata - Patrick and Reimer (1975)	40 – 80	14 – 30	7 – 9

Ecology: This species prefers high conductivity, alkaline streams (van Dam et al. 1994, Biggs and Kilroy 2000). In the present study, *E. prostratum* was found in low relative abundance in a circum-neutral reference stream of moderate conductivity (203 μS_{25}/cm).

Site number (% relative abundance):
Ref. C: 22 (1)

Family: Eunotiaceae

Genus: *Eunotia* Ehrenberg 1837

Valves are dorsiventral, typically arcuate to crescent shaped with rounded to capitate apices. Margins may be smooth or undulate. Raphe is poorly developed and difficult to resolve in LM. One rimoportula (a tube-like structure connecting the protoplast to the outer cell wall) is typically present on each valve. Terminal nodules (the internal distal raphe ends) are commonly conspicuous. Cells are solitary or may form colonies.

Eunotia bilunaris (Ehrenberg) Schaarschmidt 1880
Krammer and Lange-Bertalot (1991a): pl. 138, figs 10 – 19, p. 506

Description: Valves are arcuate with round to slightly rostrate apices. Striae are parallel and evenly spaced. Terminal nodules are resolvable in LM (Fig. 2.23). Valves are highly variable in length.

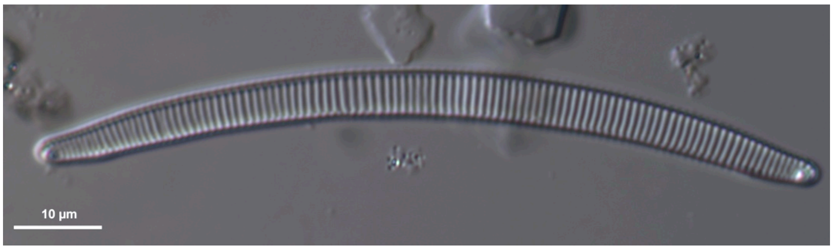


Figure 2.23. *E. bilunaris* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
E. bilunaris	31 – 85	4 – 5	15 – 17
E. bilunaris - Krammer and Lange-Bertalot (1991a)	10 – 150 (205)	1.9 – 6	(9) 11 – 28

Ecology: Van Dam et al. (1994) consider *E. bilunaris* to be pH indifferent. This species has been observed in streams receiving AMD within Malaysia (Douglas et al. 1998) as well as naturally acidic brown water streams in the U.S. (Passy et al. 2006). In the present study, *E. bilunaris* was found in greatest relative abundance in naturally acidic and moderately impacted streams.

Site number (% relative abundance):
Ref. C: 23 (< 1), 25 (< 1), 39 (< 1)
Ref. NA: 3 (42), 10 (< 1), 21 (13), 26 (< 1)
Moderate: 6 (72), 7 (19), 20 (9)
Severe: 18 (< 1)

Eunotia exigua (Brébisson ex Kützing) Rabenhorst 1864
Krammer and Lange-Bertalot (1991a): pl. 153, figs 5 – 17 p. 536

Description: Dorsal margin is convex and ventral margin is concave. Apices are capitate and deflected dorsally. Ventral margin may appear slightly undulate. Striae are parallel to slightly radiate throughout (Fig. 2.24).

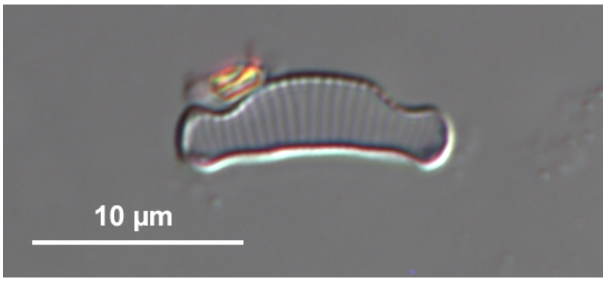


Figure 2.24. *E. exigua* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
E. exigua	13 – 19	3 – 4	21 – 24
E. exigua - Krammer and Lange-Bertalot (1991a)	(5) 8 – 28 (60)	2 (2.5) – 4 (5)	18 – 24

Ecology: This species is a common inhabitant of acidic environments (DeNicola 2000 and references within) and has been found to dominate streams impacted by AMD in the U.S. (Verb and Vis 2000, Zalack et al. 2010). In the present study, *E. exigua* was found in greatest relative abundance in both naturally acidic and moderately impacted streams.

Site(s) (% relative abundance):
Ref. C: 23 (< 1), 24 (1), 25 (2), 38 (< 1), 39 (< 1)
Ref. NA: 3 (5), 21 (2), **26** (57)
Moderate: 24 (61), 6 (8), **7** (62), **11** (56), 15 (6), 16 (8), 20 (6), **36** (91)
Severe: 18 (< 1)

Eunotia implicata Nörpel, Lange-Bertalot & Alles 1991
Krammer and Lange-Bertalot (1991a): pl. 143, figs 1 – 9A, p. 516

Description: Dorsal and ventral margin are straight. Dorsal margin constricts towards bluntly rounded apices (Fig. 2.25). Striae are parallel throughout and evenly spaced. Terminal nodules are easily identified in LM.

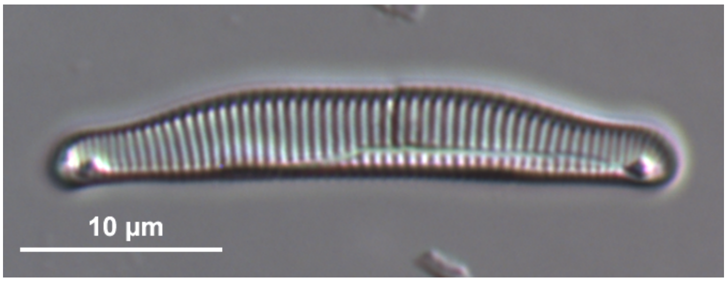


Figure 2.25. *E. implicata* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
E. implicata	26 – 38	4 –6	14 – 19
E. implicata - Krammer and Lange-Bertalot (1991a)	20 – 40	3 – 6	14 – 22

Ecology: This species prefers streams of pH < 7.0 (van Dam et al. 1994). In the Great Smoky Mountains National Park, *E. implicata* was found as an epiphyte on bryophytes in streams of pH 4.6 – 5.7 (Furey et al. 2011). In the present study, this species was found in low relative abundance in both circum-neutral reference and moderately impacted streams.

Site(s) (% relative abundance):

Ref. C: 37 (3), 38 (1)

Moderate: 4 (1), 11 (2), 15 (5), 20 (< 1)

Eunotia cf. *incisa* W. Smith ex W. Gregory 1854
Patrick and Reimer (1966): pl. 13, fig. 4, p. 232
Krammer and Lange-Bertalot (1991a): pl. 161, figs 8 – 19, p. 552

Description: Dorsal margin is convex and ventral margin is straight to slightly convex with acute apices. Striae are parallel and evenly spaced (Fig. 2.26).

Terminal nodules are distinct and offset from the apices, forming an indentation or notch on the ventral margin.

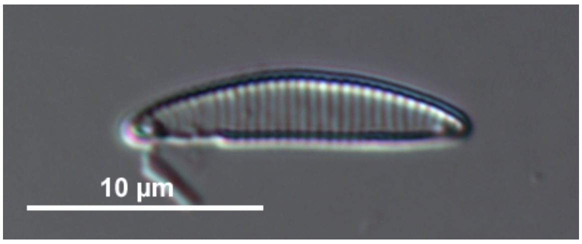


Figure 2.26. *E.* cf. *incisa* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
E. cf. *incisa*	13 – 25	2 – 4	15 – 21
E. incisa - Krammer and Lange-Bertalot (1991a)	13 – 50 (65)	(2) 4 – 6 (8)	(9) 12 – 17 (20)

Note: The valves of *E. incisa* in Krammer and Lange-Bertalot (1991a) are highly variable in shape. In the present study, *E.* cf. *incisa* most closely resembles pl. 161, fig. 9 with acutely rounded apices. In general, *E.* cf. *incisa* is narrower and of a higher striae density than *E. incisa*.

Ecology: *E. incisa* prefers acidic environments, and was found in high to moderate relative abundance in streams of pH 3.9 – 5.6 in the Great Smoky Mountains National Park (van Dam et al. 1994, Furey et al. 2011). In the present study, *E.* cf. *incisa* was found in both naturally acidic and moderately impacted streams.

Site(s) (% relative abundance):

Ref. NA: 10 (52), 21 (5)

Moderate: 20 (36)

Eunotia minor (Kützing) Grunow 1881
Krammer and Lange-Bertalot (1991a): pl. 142, figs 7 – 15, p. 514

Description: Dorsal margin is slightly constricted towards valve apices, which are bluntly rounded. Dorsal edge is smooth and convex with a straight to slightly concave ventral margin (Fig. 2.27).

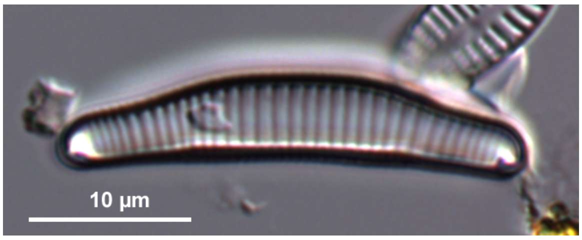


Figure 2.27. *E. minor* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
E. minor	20 – 43	5 – 7	9 – 17
E. minor - Krammer and Lange-Bertalot (1991a)	20 – 60	4.5 – 8.0	9 – 15

Ecology: This species prefers streams of pH < 7.0 (van Dam et al. 1994). In the present study, *E. minor* was found in greatest relative abundance in naturally acidic streams as well as streams moderately impacted by AMD.

Site(s) (% relative abundance):
Ref. C: 5 (< 1), 14 (< 1), 24 (2), 25 (< 1), 31 (< 1)
Ref. NA: **3** (29), 10 (4), 21 (11), 26 (< 1)
Moderate: 11 (< 1), 20 (8)
Severe: 35 (< 1)

Eunotia muscicola var. *tridentula* Nörpel & Lange-Bertalot 1993
Krammer and Lange-Bertalot (1991a): pl. 156, figs 12 – 22, p. 542

Description: Dorsal margin has more than two evenly spaced rounded humps. Ventral margin may be smooth or undulate. Striae are parallel and evenly spaced (Fig. 2.28).

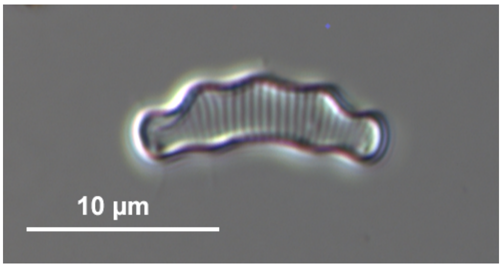


Figure 2.28. *E. muscicola* var. *tridentula* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
E. muscicola var. *tridentula*	10 – 17	3 – 4	18 – 20
E. muscicola var. *tridentula* - Krammer and Lange-Bertalot (1991a)	6 – 35	3 – 4	12 – 19

Ecology: In the Great Smoky Mountains National Park, *E. muscicola* var. *tridentula* was found in low relative abundance in streams of pH 4.2 – 5.8 (Furey et al. 2011). In the present study, this species was found in low relative abundance in circum-neutral and moderately impacted streams, and moderate abundance in a naturally acidic stream.

Site(s) (% relative abundance):
Ref. C: 24 (< 1), 25 (< 1), 31 (< 1), 39 (< 1)
Ref. NA: 26 (10)
Moderate: 11 (1), 15 (< 1), 16 (< 1)

Family: Fragilariaceae

Genus: *Diatoma* Bory de St-Vincent 1824

Diatoma is an araphid genus. Valves are elliptic to linear with rounded or capitate apices. Striae are fine and often difficult to make out under LM. The axial area is narrow and does not expand into a central area. Costae (unornamented thickening on the cell wall) run transversely across the valve face. Cells form ribbon-like or zig-zag colonies.

Diatoma mesodon (Ehrenberg) Kützing 1844

Krammer and Lange-Bertalot (1991a): pl. 99, figs 1 – 12, p. 428

Description: Valves are elliptic to lanceolate with broadly rounded apices (Fig. 2.29A). Costae are thick and prominent in LM. Axial area is very narrow. Commonly found in girdle view (Fig. 2.29B).

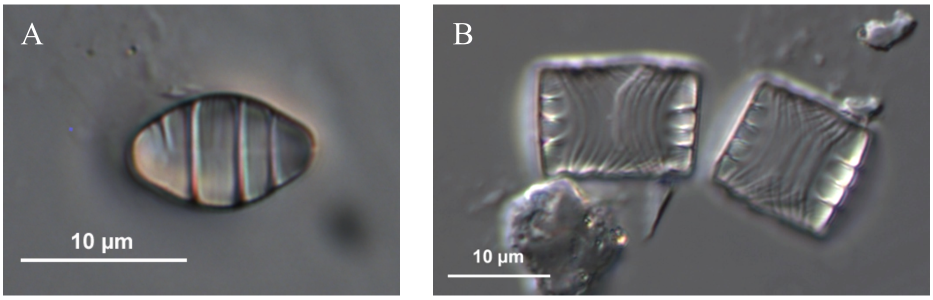


Figure 2.29. *D. mesodon* valve view (A) and girdle view (B).

Taxon	Length (µm)	Width (µm)	Costae / 10 µm
D. mesodon	12 – 14	6 – 7	2 – 4
D. mesodon - Patrick and Reimer (1966)	12 – 40	6 – 15	2 – 4

Ecology: This species is widespread throughout New Zealand and prefers circum-neutral, cold, clean streams (van Dam et al. 1994, Biggs and Kilroy 2000). In the present study, *D. mesodon* was found primarily in circum-neutral reference streams, although it was also present in low relative abundance in naturally acidic and moderately impacted streams.

Site(s) (% relative abundance):

Ref. C: 1 (< 1), 2 (3), 5 (8), 9 (16), 12 (< 1), 13 (19), 22 (2), 24 (< 1), 37 (5), **38** (41)

Ref. NA: 3 (< 1)

Moderate: 20 (2)

Diatoma tenuis C. Agardh 1812
Krammer and Lange-Bertalot (1991a): pl. 96, figs 1 – 9, p. 422

Description: Valves are linear with capitate apices that typically bend in opposite directions. Striae are very fine and cannot be seen under LM. Costae are conspicuous and unevenly spaced (Fig. 2.30).

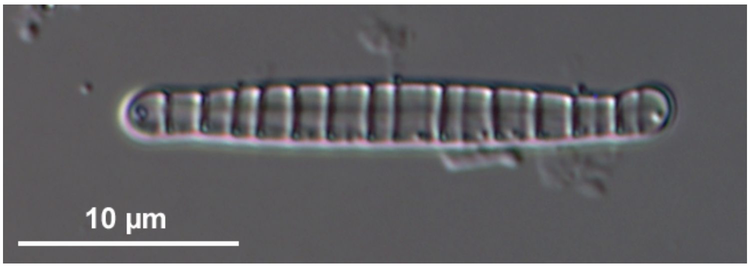


Figure 2.30. *D. tenuis* valve.

Taxon	Length (µm)	Width (µm)	Costae / 10 µm
D. tenuis	20 – 33	3 – 4	6 – 8
D. tenuis - Krammer and Lange-Bertalot (1991a)	22 – 120	2 – 5	6 – 10

Ecology: This species may be dominant in New Zealand streams, especially in the southern range of the South Island (Biggs and Kilroy 2000). It has been reported to prefer streams of conductivity near 700 µS/cm (Negro and De Hoyos 2005). In the present study, *D. tenuis* was found in highest relative abundance in a circum-neutral reference stream of moderate conductivity (203 μS_{25}/cm).

Site(s) (% relative abundance):

Ref. C: 22 (11)

Moderate: 6 (1)

Genus: *Fragilaria* Lyngbye 1819

Fragilaria is an araphid genus. Valves are linear, lanceolate or elliptic with rostrate, rounded or capitate apices. Central area of some species is slightly swollen. One rimoportula is present towards the pole of one valve. Cells are joined on the valve face by small spines forming ribbon-like colonies.

Fragilaria capucina Desmazières 1825
Patrick and Reimer (1966): pl. 3, fig. 5, p. 168
Krammer and Lange-Bertalot (1991a): pl. 108, figs 1 – 8, p. 446

Description: Valves are linear with rounded or slightly capitate apices. Central area extends across the valve face. Striae are fine and parallel throughout (Fig. 2.31). This species is variable in length, but typically found towards the smaller size range (Biggs and Kilroy 2000).

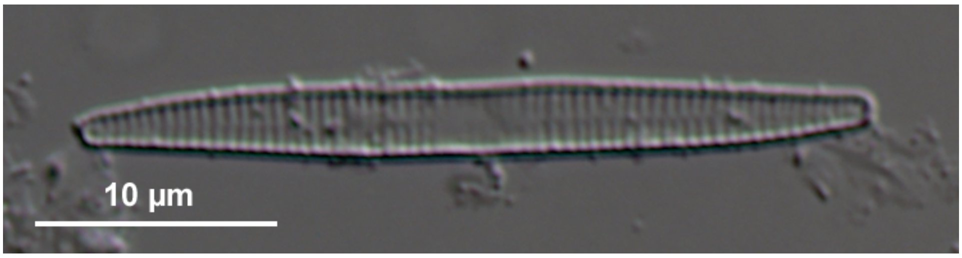


Figure 2.31. *F. capucina* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
F. capucina	24 – 36	3 – 5	14 – 18
F. capucina - Patrick and Reimer (1966)	25 – 175	2 – 5	14 – 18

Ecology: This species is widespread throughout New Zealand (Biggs and Kilroy 2000). It prefers circum-neutral streams (van Dam et al. 1994). In the present study, *F. capucina* was found primarily in circum-neutral reference streams, although it was also found in low to moderate relative abundance in streams moderately impacted by AMD.

Site(s) (% relative abundance):
Ref. C: 1 (< 1), 2 (1), 9 (< 1), 14 (< 1), **23** (30), 31 (< 1), 38 (< 1), 39 (< 1)
Ref. NA: 3 (< 1)
Moderate: 15 (6), 16 (5)

Fragilaria capucina var. *capitellata* (Grunow) Lange-Bertalot 1991
Krammer and Lange-Bertalot (1991a): pl. 109, figs 25 – 28, p. 448

Description: Valves are linear to lanceolate with capitate apices. Striae are parallel to slightly radiate towards the centre of the valve, becoming increasingly radiate towards the poles. The central area is slightly swollen and extends to the valve midline (Fig. 2.32).

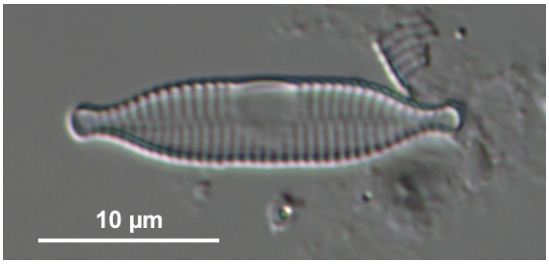


Figure 2.32. *F. capucina* var. *capitellata* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
F. capucina var. *capitellata*	13 – 23	5 – 6	17 – 20
F. capucina var. *capitellata* - Krammer and Lange-Bertalot (1991a)	10 – 100	4 – 6	18 – 20

Ecology: This species is typically found in circum-neutral lakes and ponds (Patrick and Reimer 1966, van Dam et al. 1994), although it may also dominate lotic systems (Hwang et al. 2011). In the present study, *F. capucina* var. *capitellata* was found primarily in circum-neutral streams in low to moderate relative abundance as well as one moderately impacted stream.

Site(s) (% relative abundance):

Ref. C: 9 (13), 12 (1), 13 (8), 14 (< 1), 23 (2), 25 (< 1), 37 (< 1), 38 (1)

Moderate: 4 (10)

Fragilaria capucina var. *vaucheriae* (Kützing) Lange-Bertalot 1980
Krammer and Lange-Bertalot (1991a): pl. 108, figs 10 – 15, p. 446

Description: Valves are linear to lanceolate with slightly rostrate apices. Striae are parallel or slightly radiate towards the centre of the valve, becoming increasingly radiate towards the poles. Central area spans half of the valve width and may appear slightly swollen (Fig. 2.33).

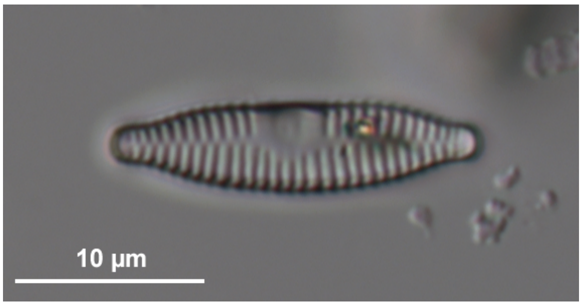


Figure 2.33. *F. capucina* var. *vaucheriae* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
F. capucina var. *vaucheriae*	18 – 30	3 – 4	12 – 15
F. capucina var. *vaucheriae* - Patrick and Reimer (1966)	10 – 40	2 – 4	12 – 16

Ecology: This species is common in New Zealand (Biggs and Kilroy 2000). It typically prefers cold streams of pH > 7 (Patrick and Reimer 1966, van Dam et al. 1994); however, it has been identified in naturally acidic New Zealand streams (Collier and Winterbourn 1990, as *Fragilaria vaucheriae*). In the present study, *F. capucina* var. *vaucheriae* was found in low to moderate relative abundance in circum-neutral reference streams, although it was also present in low relative abundance in both moderately impacted and naturally acidic streams.

Site(s) (% relative abundance):
Ref. C: 1 (1), 2 (11), 8 (3), 9 (10), 12 (2), 14 (1), 22 (2), 23 (< 1), 24 (1), 25 (6), 37 (< 1), 38 (5), 39 (2)
Ref. NA: 10 (< 1)
Moderate: 4 (< 1), 6 (< 1), 11 (< 1), 15 (< 1), 16 (< 1)

Genus: *Fragilariforma* D.M. Williams & Round 1988

Fragilariforma is an araphid genus. Valves are linear, lanceolate or elliptic with capitate to rostrate apices. Axial area is very narrow and a central area is absent.

One rimoportula is present near the pole of one valve. *Fragilariforma* cells are joined by small spines between striae, forming linear or zig-zag colonies.

Fragilariforma virescens (Ralfs) D.M. Williams & Round 1988
Krammer and Lange-Bertalot (1991a): pl. 126, figs 1 – 10, p. 482 as *Fragilaria virescens* Ralfs 1843

Description: Valves are elliptic (Fig. 2.34A) to linear (Fig. 2.34B) with rostrate apices. Axial area is very narrow, resulting in striae that appear to extend across valve face. Striae are fine and dense.

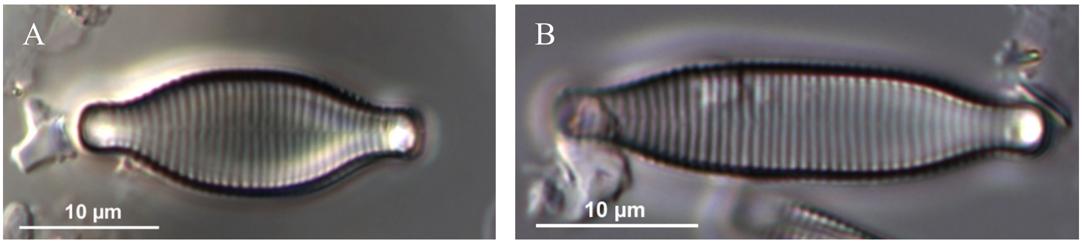


Figure 2.34. *F. virescens* elliptic (A) and linear (B) valve shapes. Note the fine striae that appear to extend across the valve face.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
F. virescens	24 – 60	6 – 8	14 – 17
Fragilaria virescens - Patrick and Reimer (1966)	12 – 120	5 – 10	15 – 19

Ecology: This species prefers circum-neutral, low conductivity streams (van Dam et al. 1994, Cox 1996). In the present study, *F. virescens* was found in greatest relative abundance in a circum-neutral and naturally acidic reference stream.

Site(s) (% relative abundance):
Ref. C: 24 (2), 25 (< 1)
Ref. NA: 26 (2)
Moderate: 11 (< 1)

Genus: *Ulnaria* (Kützing) P. Compère 2001

Ulnaria is an araphid genus. Valves are linear to lanceolate with variable apices. Striae are parallel. Axial area is narrow and opens to a rectangular or square central area. Two rimoportulae are present on each valve, one near each pole. Cells are typically attached at one pole by a mucilage pad, forming radiate colonies.

Ulnaria acus (Kützing) M. Aboal 2003
Krammer and Lange-Bertalot (1991a): pl. 120, fig. 2, p. 470 as *Synedra acus* Kützing 1844

Description: Valves are linear to lanceolate with rounded to capitate apices. Striae are parallel throughout. Axial area is narrow, widening slightly towards the centre of the valve. Central area is rectangular and longer than it is wide. Striae may be faintly identified within the central area ('ghost striae') (Fig. 2.35).

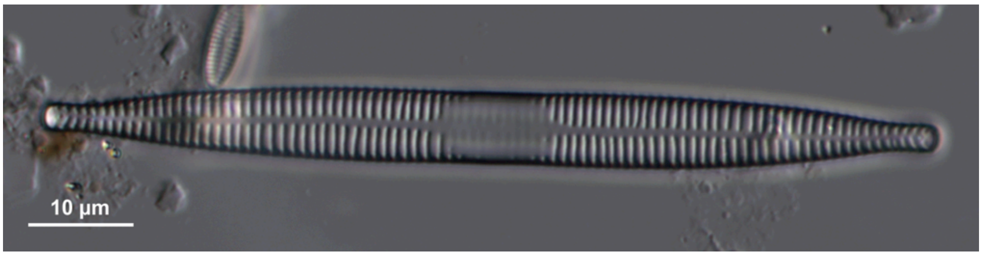


Figure 2.35. *U. acus* valve. Note the central area that is longer than it is wide.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
U. acus	92 – 115	6 – 7	11 – 12
Synedra acus - Patrick and Reimer (1966)	90 – 180	4.5 – 6	11 – 14

Ecology: This species prefers circum-neutral environments of moderate conductivity (Patrick and Reimer 1966). In the present study, *U. acus* was found in low relative abundance in circum-neutral reference streams as well as one moderately impacted stream.

Site(s) (% relative abundance):

Ref. C: 13 (< 1), 22 (< 1), 31 (1)

Moderate: 6 (< 1)

Family: Gomphonemataceae

Genus: *Gomphonema* Ehrenberg 1832

Valves are heteropolar (asymmetrical across the transapical axis), either linear or lanceolate in shape with rostrate to capitate apices. Raphe is typically slightly sinuous. A single stigma is commonly present in the central area of the valve. One or more striae opposite the stigma may be either short or absent. *Gomphonema* cells are colonial and stalked.

Gomphonema acuminatum Ehrenberg 1832
Krammer and Lange-Bertalot (2008): pl. 160, figs 1 – 12, p. 762

Description: Valves are clavate, swollen at the head pole (the wider pole) and in the centre. A single stigma is located in the centre of the valve across from one or more shortened striae. The raphe is slightly sinuous and lies in a narrow, linear axial area (Fig. 2.36).

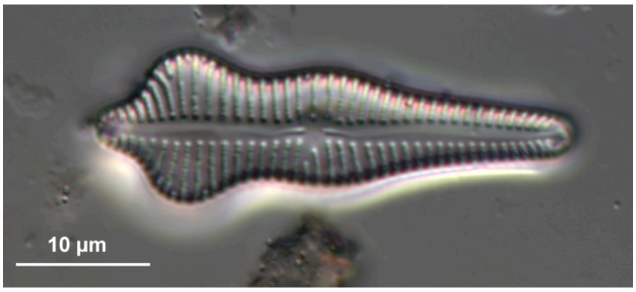


Figure 2.36. *G. acuminatum* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
**G. acuminatum*	35	8	12
G. acuminatum - Kelly et al. (2005)	20 – 120	5 – 17	8 – 13

*Fewer than 10 valves were measured.

Ecology: This species is widespread throughout New Zealand (Biggs and Kilroy 2000). It typically prefers slightly alkaline environments (van Dam et al. 1994). In the present study, *G. acuminatum* was found in low relative abundance in a single moderately impacted stream.

Site(s) (% relative abundance):

Moderate: 6 (< 1)

Gomphonema angustatum (Kützing) Rabenhorst 1864
Krammer and Lange-Bertalot (2008): pl. 155, figs 1 – 21, p. 752

Description: Valves are clavate to lanceolate with rostrate apices. Axial area is narrow. The distance from the central striae and the adjacent striae is noticeably wider than the distance between striae throughout the valve, which distinguishes this species from *Gomphonema parvulum*. Striae radiate throughout (Fig. 2.37).

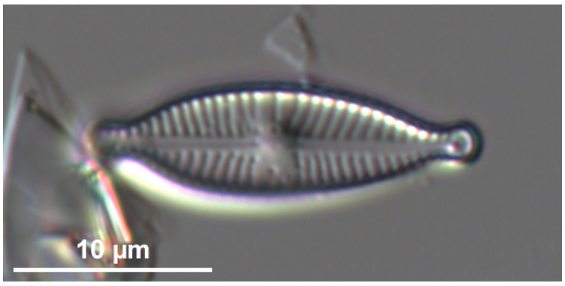


Figure 2.37. *G. angustatum* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
G. angustatum	21 – 26	6 – 7	12 – 14
G. angustatum - Kelly et al. (2005)	12 – 45	5 – 9.5	7 – 14

Ecology: This species is sensitive to organic pollution (Kelly and Whitton 1995). It may tolerate streams of moderate conductivity (optimum: 264 µS/cm) (Potapova and Charles 2003). In the present study, *G. angustatum* was found in low relative abundance in circum-neutral reference and one moderately impacted stream.

Site(s) (% relative abundance):

Ref. C: 8 (< 1), 22 (< 1), 24 (5), 25 (< 1), 31 (< 1)

Moderate: 11 (< 1)

Gomphonema angustum C. Agardh 1831

Krammer and Lange-Bertalot (1991b): pl. 84, figs 9 – 14, p. 416

Description: Valves are linear to clavate with rounded apices and a slightly swollen centre. A single stigma is present across from shortened striae on either side on the valve. Axial area is wide and linear. Striae are parallel towards the centre of the valve and radiate towards the poles (Fig. 2.38).

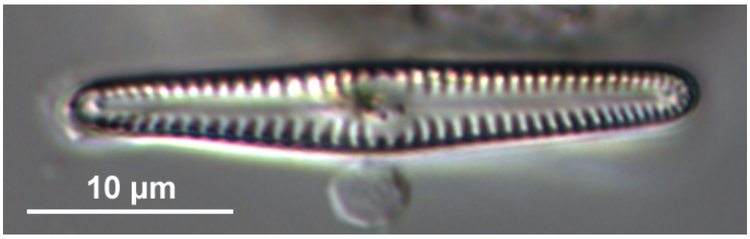


Figure 2.38. *G. angustum* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
G. angustum	28 – 34	5 – 6	10 – 12
G. angustum - Kelly et al. (2005)	12 – 130	3 – 12	9 – 12

Ecology: This species is widespread throughout New Zealand and prefers un-polluted streams of pH > 7 (van Dam et al. 1994, Biggs and Kilroy 2000). In the present study, *G. angustum* was found exclusively in circum-neutral reference streams.

Site(s) (% relative abundance):

Ref. C: 1 (2), 2 (2), 8 (< 1), 12 (< 1), 14 (< 1)

Gomphonema clavatum Ehrenberg 1832

Krammer and Lange-Bertalot (1991b): pl. 83, figs 1 – 3, p. 414

Description: Valves are clavate with rounded apices. Raphe is noticeably sinuous. A single stigma is present opposite one or two shortened striae in the central area. Striae are coarse and present on both sides of the central area (Fig. 2.39).

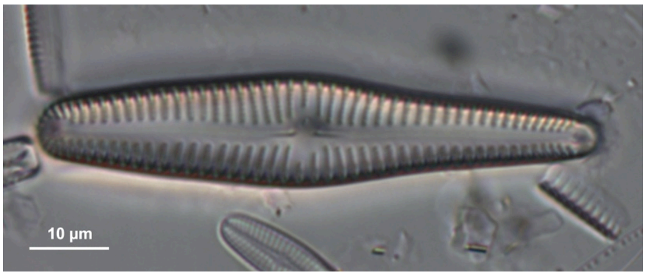


Figure 2.39. *G. clavatum* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
G. clavatum	42 – 72	8 – 12	10 – 12
G. clavatum - Kelly et al. (2005)	20 – 95	6 – 14	9 – 15

Ecology: This species prefers oxygen-rich, circum-neutral streams (van Dam et al. 1994, Fore and Grafe 2002, Andrén and Jarlman 2008). In the present study, *G. clavatum* was typically found in circum-neutral streams in low to moderate relative abundance.

Site(s) (% relative abundance):
Ref C: 1 (3), 2 (< 1), 8 (< 1), 9 (< 1), 23 (< 1), 25 (< 1), 37 (10), 28 (< 1)
Moderate: 16 (< 1)

Gomphonema gracile Ehrenberg 1838
Patrick and Reimer (1975): pl. 17, figs 1 – 3, p. 158.

Description: Valves are lanceolate and only slightly heteropolar with rounded apices. Striae are coarse and parallel to slightly radiate in the centre of the valve, increasingly radiate towards the poles. Central area is formed by one shortened stria. A single stigma is present in the central area across from the shortened stria. Axial area is narrow and linear (Fig. 2.40).

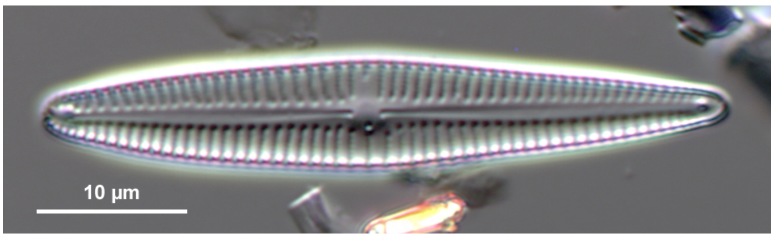


Figure 2.40. *G. gracile* valve.

Taxon	Length (μm)	Width (μm)	Striae / 10 μm
**G. gracile*	35 – 45	6 – 8	11
G. gracile - Kelly et al. (2005)	20 – 100	4 – 11	9 - 17

*Fewer than 10 valves were measured.

Ecology: This species typically prefers circum-neutral streams (van Dam et al. 1994), although it may tolerate a wide range of pH (Patrick and Reimer 1975). In the present study, *G. gracile* was found in low relative abundance in both acidic (natural and moderate AMD) and circum-neutral streams.

Site(s) (% relative abundance):
Ref. C: 9 (1)
Ref. NA: 3 (1)
Moderate: 15 (3), 36 (< 1)

Gomphonema minutum (C. Agardh) C. Agardh 1831
Krammer and Lange-Bertalot (1991b): pl. 81, figs 1 – 23, p. 410

Description: Valves are clavate with rounded apices. A single stigma is present across from a shortened stria on either side of the valve forming a rectangular central area. Striae are slightly radiate towards the valve centre, becoming more so towards the poles (Fig. 2.41).

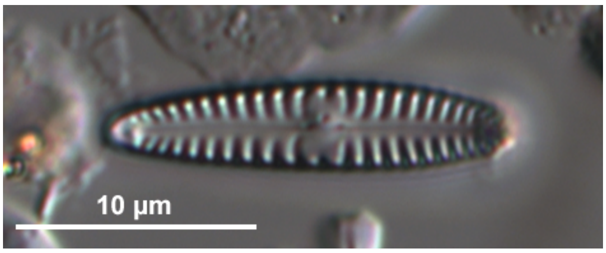


Figure 2.41. *G. minutum* valve.

Taxon	Length (μm)	Width (μm)	Striae / 10 μm
G. minutum	15 – 20	4 – 5	12 – 14
G. minutum - Patrick and Reimer (1975)	15 – 25	4 – 6	11 – 13

Ecology: This species is widespread and common throughout New Zealand (Biggs and Kilroy 2000). It prefers clean, circum-neutral streams (van Dam et al. 1994, Biggs and Kilroy 2000). In the present study, *G. minutum* was found exclusively in circum-neutral reference streams in low to high relative abundance.

Site(s) (% relative abundance):
Ref. C: 1 (3), 2 (9), 5 (< 1), **8** (54), 9 (< 1), 12 (17), 13 (4), **14** (21), 22 (4), 24 (3), 37 (3), 39 (< 1)

Gomphonema parvulum (Kützing) Kützing 1849
Krammer and Lange-Bertalot (1991b): pl. 76, figs 1 – 29, p. 400

Description: Valves are clavate to lanceolate with rostrate apices. A single stigma is present across from a shortened stria forming the central area. Axial area is narrow and linear. Striae are typically parallel throughout the valve. The space between the central shortened stria and the adjacent striae is only slightly wider than the distance between striae throughout the valve (Fig. 2.42).

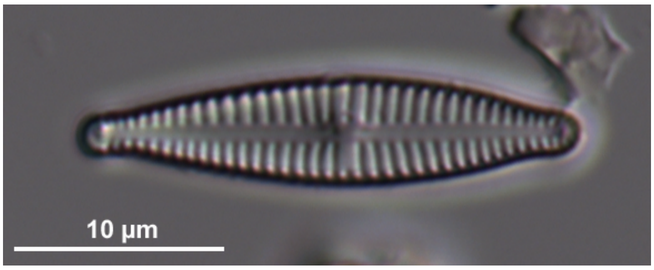


Figure 2.42. *G. parvulum* valve. Note the evenly spaced striae.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
G. parvulum	18 – 25	5 – 6	13 – 16
G. parvulum - Patrick and Reimer (1975)	15 – 30	5 – 8	13 – 16

Ecology: Often considered a "weedy" species, *G. parvulum* is able to tolerate a variety of environmental conditions, including high metals and organic pollution (Kelly and Whitton 1995, Ivorra et al. 2002). In the present study, this species was typically found in circum-neutral reference streams in low to high relative abundance. It was also present in low abundance in moderately impacted and naturally acidic streams.

Site(s) (% relative abundance):
Ref. C: **1** (52), 2 (13), 5 (6), 8 (13), **9** (23), 12 (4), 13 (7), 14 (5), **22** (21), 23 (9), 24 (9), 25 (< 1), 31 (2), 37 (3), 38 (< 1), 39 (3)
Ref. NA: 3 (1)
Moderate: 4 (4), 6 (< 1), 7 (3), 11 (< 1), 15 (4), 16 (< 1), 36 (< 1)

Genus: *Reimeria* J.P. Kociolek & E.F. Stoermer 1987

Valves are linear to lanceolate with rounded apices. Dorsal margin is convex and ventral margin is typically straight with a gibbous central area where striae are absent. A single stigma is present near the proximal raphe endings. *Reimeria* is a free-living, commonly epilithic genus.

Reimeria sinuata (Gregory) Kociolek & Stoermer 1987
Krammer and Lange-Bertalot (2008): pl. 148, figs 10 – 17, p. 738 as *Cymbella sinuata* W. Gregory 1856

Description: Valves are elliptic with rounded apices. One stigma is located near the proximal raphe endings. Striae are radiate and may appear slightly curved. A bulge devoid of striae is present on the ventral side of the valve (Fig. 2.43).

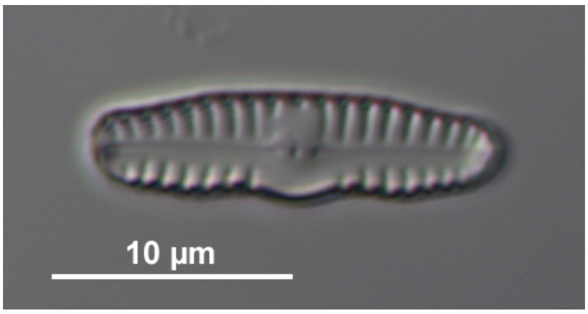


Figure 2.43. *R. sinuata* valve. Note the bulge on the ventral margin.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
R. sinuata	15 – 20	3 – 5	10 – 13
R. sinuata - Kelly et al. (2005)	9 – 40	3.5 – 9	8 – 16

Ecology: This species is widespread throughout New Zealand (Biggs and Kilroy 2000). It prefers circum-neutral streams and is tolerant of heavy organic pollution (van Dam et al. 1994, Kelly and Whitton 1995). In the present study, *R. sinuata* was found in low relative abundance in circum-neutral reference streams.

Site(s) (% relative abundance):
Ref. C: 13 (1), 22 (< 1), 37 (1)

Family: Naviculaceae

Genus: *Navicula* Bory de Saint-Vincent 1822

Valves are lanceolate to linear with rounded, rostrate, or capitate apices. Striae typically radiate near the centre and become convergent at poles. The raphe is central with proximal ends slightly deflected. Cells are solitary and typically seen in valve view.

Navicula cf. *angusta* Grunow 1860
Lange-Bertalot (2001): pl. 2, figs 1 – 8, p. 241

Description: Valves are linear with rounded, slightly protracted apices. Axial area is narrow and linear, opening to a circular central area. Striae radiate near the centre, becoming convergent towards the poles (Fig. 2.44).

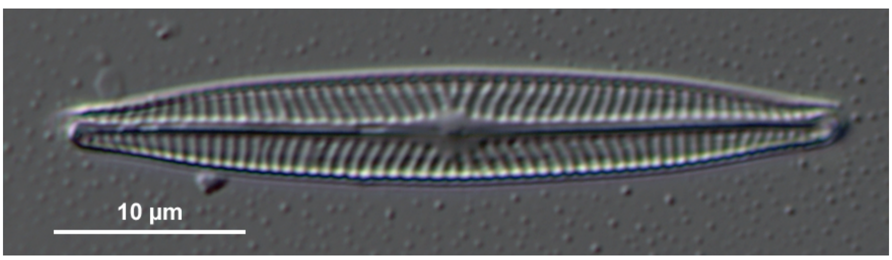


Figure 2.44. *N.* cf. *angusta* valve. Note linear valve shape.

Note: Differs from *Navicula angusta* in the shape of the central area. *N.* cf. *angusta* has a circular central area, whereas *N. angusta* has a noticeably asymmetrical central area.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
N. cf. *angusta*	35 –51	6 – 8	12 – 13
N. angusta - Lange-Bertalot (2001)	30 – 78	5 – 8	11 – 12

Ecology: *Navicula angusta* is typically found in slightly acidic streams (van Dam et al. 1994). In the present study, *N.* cf. *angusta* was found exclusively in circum-neutral reference streams in low to moderate relative abundance.

Site(s) (% relative abundance):
Ref. C: 2 (< 1), 12 (8), 14 (9), 24 (< 1), 25 (< 1), 38 (4)

Navicula lanceolata Ehrenberg 1838
Patrick and Reimer (1966): pl. 48, figs 20 – 21, p. 560

Description: Valves are lanceolate with slightly protracted, broadly rounded apices. Striae radiate towards the centre of the valve and become convergent near the poles. Striae may appear slightly curved. Axial area is narrow and opens to a distinctly oval central area (Fig. 2.45).

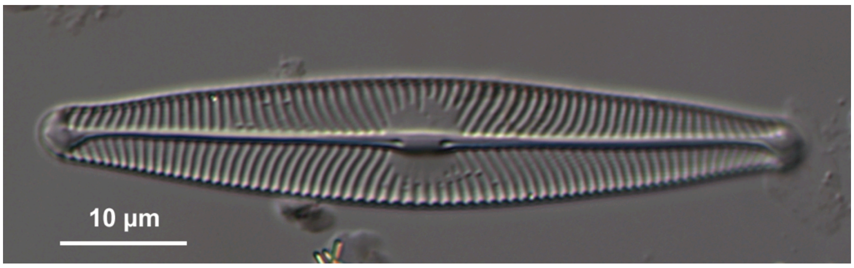


Figure 2.45. *N. lanceolata* valve. Note the oval central area.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
N. lanceolata	40 – 57	9 – 10	12 – 13
N. lanceolata - Lange-Bertalot (2001)	27 – 70	6 – 12	10 –13

Ecology: *N. lanceolata* is widespread and common throughout New Zealand, especially in streams of low to moderate conductivity (Biggs and Kilroy 2000). It prefers slightly alkaline environments and can tolerate heavy organic pollution (van Dam et al 1994, Kelly and Whitton 1995). In the present study, *N. lanceolata* was found primarily in circum-neutral reference streams. A single valve was also found in one moderately impacted stream.

Site(s) (% relative abundance):
Ref. C: 2 (2), 13 (< 1), 22 (2), 24 (1), 39 (< 1)
Moderate: 16 (< 1)

Navicula radiosafallax Lange-Bertalot 1993
Patrick and Reimer (1966): pl. 48, fig. 16, p. 560 as *Navicula radiosa* var. *parva* Wallace 1960

Description: Valves are lanceolate with rounded apices. Apices are not protracted. Striae radiate towards centre of the valve and are convergent near the poles. Axial area is narrow and linear, opening to an elliptical central area (Fig. 2.46).

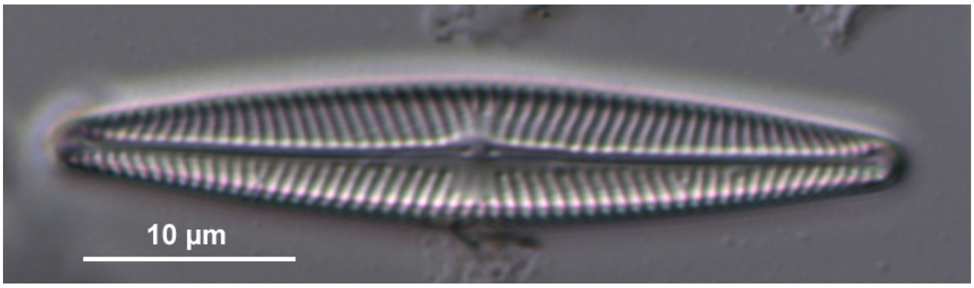


Figure 2.46. *N. radiosafallax* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
N. radiosafallax	34 – 39	6 – 7	13 – 14
N. radiosafallax - Lange-Bertalot (2001)	30 – 50	5.5 – 6.6 (7)	13 – 14

Ecology: Little is known regarding the ecology of this species (Lange-Bertalot 2001). In the present study, *N. radiosafallax* was primarily found in circum-neutral reference streams in low relative abundance.

Site(s) (% relative abundance):

Ref. C: 9 (2), 25 (2), 37 (1), 39 (< 1)

Ref. NA: 26 (< 1)

Moderate: 15 (3)

Navicula rhynchocephala Kützing 1844
Patrick and Reimer (1966): pl. 48, fig. 6, p. 560
Lange-Bertalot (2001): pl. 9, figs 6 – 10, p. 254

Description: Valves are lanceolate with distinctly protracted, rostrate apices. Striae radiate towards the centre of the valve and become convergent near the poles. Striae are more widely spaced in the centre of the valve. Axial area is narrow opening to a circular, often asymmetrical central area (Fig. 2.47).

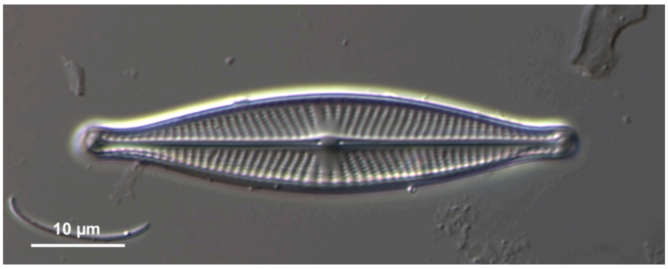


Figure 2.47. *N. rhynchocephala* valve. Note the protracted apices.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
N. rhynchocephala	48 – 55	9 – 10	10 – 12
N. rhynchocephala - Patrick and Reimer (1966)	35 – 60	9 – 14	8 – 12

Ecology: This species is typically found in slightly alkaline environments and can tolerate moderate levels of organic pollution (van Dam et al. 1994, Biggs and Kilroy 2000). In the present study, *N. rhynchocephala* was found in low relative abundance in circum-neutral streams as well as one moderately impacted stream.

Site(s) (% relative abundance):

Ref. C: 9 (< 1), 25 (< 1), 39 (< 1)

Moderate: 15 (< 1)

Family: Pinnulariaceae

Genus: *Pinnularia* Ehrenberg 1843

Valves are linear, lanceolate, or elliptic with rostrate to capitate apices. Valve margins may be straight or slightly undulate. Striae are typically chambered. Proximal raphe endings are expanded and deflected in the same direction. Distal raphe ends are hooked in most species, forming a question mark shape. Cells are solitary.

Pinnularia cf. *acidophila* Hofmann & K. Krammer 2000
Krammer (2000): pl. 14, figs 1 – 22, p. 286.

Description: Valves are linear to slightly lanceolate with rounded apices. Axial area is narrow, gradually widening to a broad, rectangular central area that extends to the valve margins. Striae radiate at the centre and abruptly become convergent, forming a diamond-shaped pattern approximately halfway to the poles (Fig. 2.48A, B). This pattern is also present on cell margins in girdle view (Fig. 2.48C). Valves may not fully disassociate after acid cleaning and remain attached at one pole in girdle view (Fig. 2.48D).

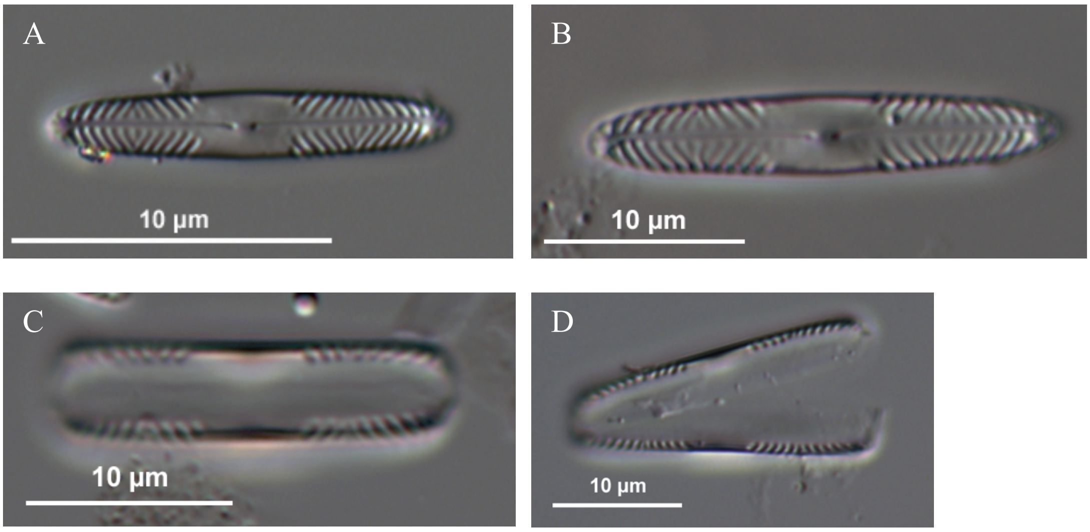


Figure 2.48. *P*. cf. *acidophila* valve view (A, B), girdle view (C), and girdle view, detached at one pole (D). Note the abrupt change in striae orientation.

Note: Differs from *P. acidophila* in valve shape, as well as width in girdle view. Krammer (2000) noted that *P. acidophila* is characterised by wide frustules in girdle view due to large girdle bands, but does not provide specific measurements. In the present study, *P.* cf. *acidophila* was found in a wide range of widths in girdle view, from 3 – 7 µm. It was also typically wider in valve view than *P. acidophila.* Another species, *Pinnularia acoricola* Hustedt, is similar in size and striae arrangement to *P.* cf. *acidophila*. However, valves of *P. acoricola* are more lanceolate in shape than *P.* cf. *acidophila*.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
P. cf. *acidophila*	14 – 22	3 – 4	14 – 16
P. acidophila - Krammer (2000)	12 – 22	3 – 3.3	13 – 16

Ecology: *Pinnularia acidophila* has been described from lakes receiving AMD in Germany (Krammer 2000) and Korea (Kim et al. 2008). In the present study, *P.* cf. *acidophila* dominated all severely impacted streams at a relative abundance of up to 100%.

Site(s) (% relative abundance):
Ref C: 12 (< 1), 38 (< 1)
Ref. NA: 3 (< 1), 10 (< 1), 26 (< 1)
Moderate: 15 (2), **16** (45), 20 (3), 36 (4)
Severe: 17 (69), **18** (91), **19** (92), **27** (86), **28** (100), **29** (100), **30** (100), **32** (99), **33** (100), **34** (99), **35** (99)

Pinnularia cf. *amabilis* K. Krammer 2000
Krammer (2000): pl. 86, figs 1 – 9, p. 430

Description: Valves are linear with capitate apices. Axial area is narrow, widening to a broad, rhomboidal central area that extends to the valve margins. Striae radiate towards the centre and become strongly convergent towards the poles. Distal raphe endings are hooked and clearly visible in LM (Fig. 2.49).

Figure 2.49. *P.* cf. *amabilis* valve.

Note: Krammer (2000) describes *P. amabilis* as having curved striae throughout the length of valve. In the present study, striae of *P.* cf. *amabilis* were linear, occasionally curved near the poles.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
P. cf. *amabilis*	29 – 44	6 – 9	11 – 14
P. amabilis - Krammer (2000)	32 – 53	6.5 – 8	10 – 12

Ecology: *Pinnularia amabilis* is typically found in oligotrophic streams of moderate conductivity (Krammer 2000). In the present study, *P.* cf. *amabilis* was found in low relative abundance in circum-neutral, naturally acidic and moderately impacted streams.

Site(s) (% relative abundance):
Ref. C: 9 (< 1), 13 (< 1), 24 (< 1), 39 (< 1)
Ref. NA: 21 (< 1), 26 (2)
Moderate: 6 (< 1), 11 (1), 15 (2), 16 (< 1), 20 (< 1)

Family: Rhoicospheniaceae

Genus: *Rhoicosphenia* Grunow 1860

Cells are wedge shaped in valve and girdle view, with one convex and one concave valve. The raphe of the concave valve is fully developed. The raphe of the convex valve is present as small slits near the poles. *Rhoicosphenia* is a stalked, commonly epiphytic genus.

Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot 1980

Krammer and Lange-Bertalot (2008): pl. 91, figs 20 – 28, p. 622

Description: Valves are clavate with bluntly to narrowly rounded apices. On the raphe valve, striae are wider in the centre than near the poles and slightly radiate. On the rudimentary raphe valve, striae are parallel (2.50A). Axial area is narrow and central area is absent on both valves. Commonly seen in girdle view (2.50B).

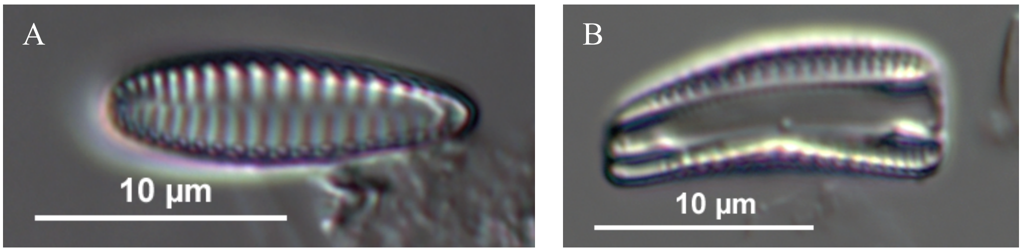


Figure 2.50. *R. abbreviata* rudimentary raphe valve (A) and girdle view (B).

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
R. abbreviata	15 – 30	4 – 8	12 – 18
R. abbreviata - Patrick and Reimer (1966)	12 – 75	4 – 8	9 – 20

Ecology: This species is common in New Zealand and is tolerant of organic pollution (Biggs and Kilroy 2000, Potapova and Charles 2007). In the present study, *R. abbreviata* was found in low relative abundance in both circum-neutral and moderately impacted streams.

Site(s) (% relative abundance):

Ref. C: 37 (1)

Moderate: 16 (3), 20 (< 1)

Family: Rhopalodiaceae

Genus: *Epithemia* Kützing 1844

Valves are dorsiventral with bluntly rounded, rostrate or capitate apices. Transapical costae are conspicuous. Raphe is eccentric and located near the ventral margin. Proximal raphe endings are deflected towards the distal margin. Cells are solitary and commonly epiphytic. *Epithemia* species are typically found in girdle view.

Epithemia adnata (Küzing) Brébisson 1838
Krammer and Lange-Bertalot (1997): pl. 107, figs 1 – 11, p. 107

Description: Valves are crescentic with a convex dorsal margin and a concave ventral margin. Apices are broadly rounded. Costae are thick and irregularly spaced. The raphe curves upwards towards the centre, barely reaching the valve midline (Fig. 2.51).

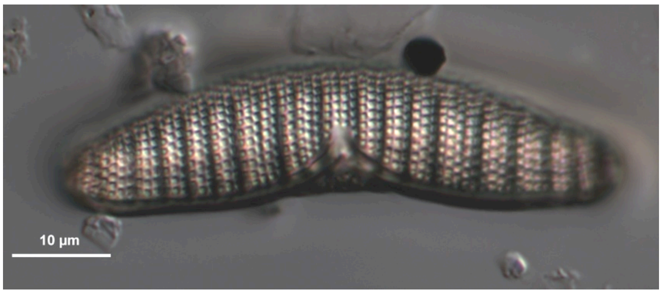


Figure 2.51. *E. adnata* valve.

Taxon	Length (µm)	Width (µm)	Costae / 10 µm
**E. adnata*	72 – 82	9 – 14	3 – 4
E. adnata - Krammer and Lange-Bertalot (1997)	15 – 150	7 – 14	2 – 8

*Fewer than 10 valves were measured.

Ecology: This species typically inhabits streams of moderate to high conductivity (Biggs and Kilroy 2000, Potapova and Charles 2003). It may be found throughout New Zealand but is not usually dominant (Biggs and Kilroy 2000). In the present study, *E. adnata* was found in low relative abundance in a circum-neutral reference and moderately impacted stream.

Site(s) (% relative abundance):
Ref. C: 14 (2)

Moderate: 16 (< 1)

Epithemia sorex Kützing 1844
Krammer and Lange-Bertalot (1997): pl. 106, figs 1 – 13, p. 428

Description: Valves are semicircular with a smooth, convex dorsal margin and a smooth, slightly concave ventral margin. Apices are capitate and curved dorsally. Raphe curves towards the centre of the valve, nearly reaching the dorsal margin. Costae are evenly spaced and distinct in LM (Fig. 2.52).

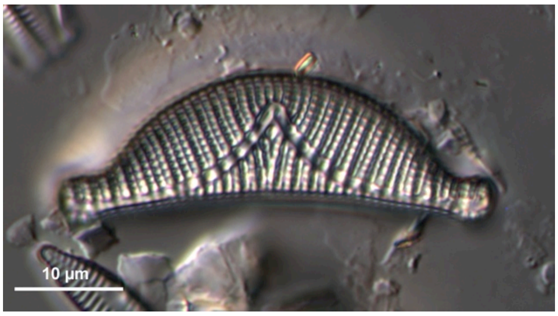


Figure 2.52. *E. sorex* valve.

Taxon	Length (µm)	Width (µm)	Costae / 10 µm
**E. sorex*	39 – 42	11	6
E. sorex - Krammer and Lange-Bertalot (1997)	20 – 65	6 – 15	5 – 7

*Fewer than 10 valves were measured.

Ecology: This species is common in enriched, lowland streams within New Zealand (Biggs and Kilroy 2000). It typically prefers high conductivity streams (Patrick and Reimer 1975). In the present study, *E. sorex* was found in low relative abundance at one circum-neutral reference stream.

Site(s) (% relative abundance):
Ref. C: 2 (< 1)

Family: Surirellaceae

Genus: *Surirella* Turpin 1828

Valves are linear to elliptic with rounded, rostrate to cuneate apices. Frustules are isopolar or heteropolar and are typically highly silicified. Raphe is on a keel (an elevated ridge) and runs along the margins of the valve face. Cells are solitary. *Surirella* is primarily an epipelic genus.

Surirella angusta Kützing 1844
Krammer and Lange-Bertalot (1997): pl. 133, figs 6 – 13, p. 486

Description: Valves are linear to elliptic with cuneate apices (Fig. 2.53). Frustules are isopolar. Striae are fine and difficult to resolve in LM. Axial area is very narrow and linear. Central area is absent.

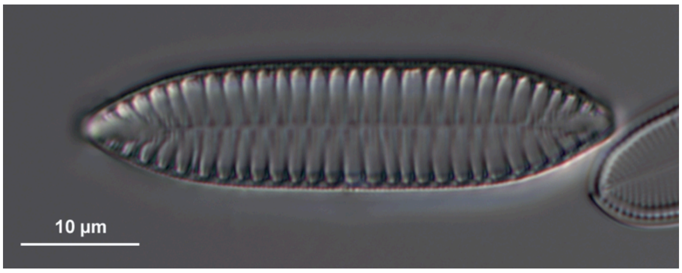


Figure 2.53. *S. angusta* valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm	Fibulae / 10 µm
S. angusta	30 – 40	9 – 13	UR	7 – 9
S. angusta - Krammer and Lange-Bertalot (1997)	18 – 70	6 – 15	22 – 28	7 – 8

Ecology: This species is widespread throughout New Zealand, especially in organically polluted lowland streams (Biggs and Kilroy 2000). *Surirella* is a highly motile genus and may indicate elevated siltation levels (Hill et al. 2001). In the present study, *S. angusta* was found primarily in circum-neutral reference and moderately impacted streams in low to moderate relative abundance.

Site(s) (% relative abundance):

Ref. C: 2 (3), 12 (5), 13 (< 1), 24 (< 1), 31 (< 1)

Ref. NA: 3 (< 1)

Moderate: 6 (< 1), 7 (5), 11 (15), 15 (< 1), 16 (1), 20 (< 1), 36 (2)

Family: Tabellariaceae

Genus: *Tabellaria* Ehrenberg ex Kützing 1844

Tabellaria is an araphid genus. Cells are rectangular in girdle view and linear in valve view, with a swollen centre and poles. Striae are typically irregularly spaced and may be parallel or radiate from the centre of the valve. A single rimoportula is present near the centre of the valve. Cells form zig-zag chains.

Tabellaria flocculosa (Roth) Kützing 1844
Patrick and Reimer (1966): pl. 1, figs 4 – 5, p. 164
Krammer and Lange-Bertalot (1991a): pl. 106, figs 1 – 13, p. 442

Description: Valve is slightly asymmetrical to the transverse and/or apical axes. The valve is swollen in the centre, where it is wider than either pole. A single rimoportula is present in the centre of the valve and may be seen in LM under high magnification. Striae are parallel and irregularly spaced (Fig. 2.54A). Isolated girdle bands are often observed in a cleaned sample (Fig. 2.54B).

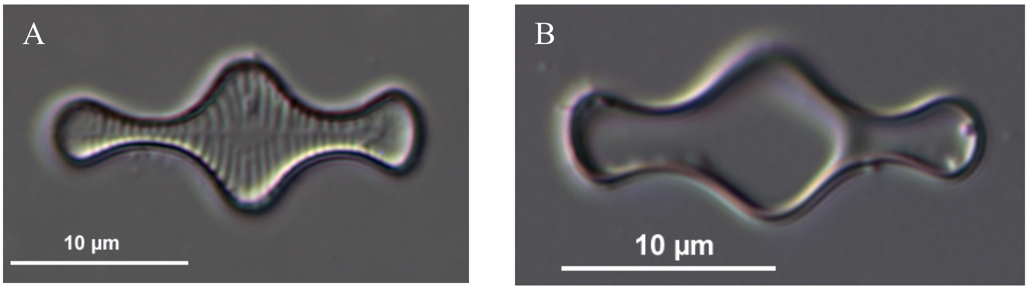


Figure 2.54. *T. flocculosa* valve (A) and an isolated girdle band (B). Note the rimoportula that appears as a small depression near the centre of the valve.

Taxon	Length (µm)	Width (µm)	Striae / 10 µm
T. flocculosa	18 – 21	7 – 9	15 – 18
T. flocculosa - Krammer and Lange-Bertalot (1991a)	6 – 130	3.8 – 8.5	13 – 20

Ecology: *T. flocculosa* is the dominant *Tabellaria* species in New Zealand (Biggs and Kilroy 2000). It can tolerate a range of water chemistries although it has been reported as sensitive to metal pollution (Hirst et al. 2002). *T. flocculosa* is acid-tolerant and prefers streams with pH near 5.5 (van Dam et al. 1994). In the present study, relative abundance was low to high in circum-neutral reference streams, and low in both naturally acidic and moderately impacted streams.

Site(s) (% relative abundance):
Ref. C: 9 (< 1), **23** (32), 24 (< 1), **25** (47), 38 (3), 39 (< 1)
Ref. NA: 3 (< 1), 10 (1)
Moderate: 4 (2), 6 (< 1), 15 (3)

Chapter 3

Diatom communities along an acid mine drainage gradient

3.1. Introduction

Acid mine drainage (AMD) associated with mineral extraction is a significant and increasing environmental issue globally. AMD is generated when coal, coal tailings, or overburden are exposed to air and water, which oxidizes sulphide minerals (commonly pyrite), produces sulphuric acid and releases associated heavy metals (Kelly 1988). In New Zealand, coal mining has occurred on the West Coast of the South Island since 1872, where an estimated 125 kilometres of streams are impacted by AMD (James 2003, Harding and Boothroyd 2004). Much of this coal occurs within the Brunner Coal Measures, which are rich in sulphates (Pope et al. 2010). Streams receiving AMD within the region are typically characterised by low pH (e.g. < 3), high concentrations of metals (mostly iron, aluminium, and manganese) and high specific conductivity (Akcil and Koldas 2006, Pope et al. 2010). AMD can contaminate both surface runoff and groundwater, impacting freshwater systems across a catchment (Novis and Harding 2007). Dissolved metals may precipitate as pH increases downstream from the source of contamination, covering the streambed in metal hydroxide deposits, typically iron or aluminium. AMD has the potential to substantially alter the abundance, composition, and biomass of freshwater communities, both through chemical (dissolved metals, low pH) and physical (metal oxide deposition) mechanisms (Niyogi et al. 2002, Pond et al. 2008, Greig et al. 2010).

In addition to anthropogenically acidified streams, large areas of the West Coast contain naturally acidic streams (pH 4.3 – 5.7). This natural acidity is a result of fulvic and humic organic acids leaching into streams from the surrounding podocarp rainforest and swampy peatland (Collier et al. 1990). While naturally acidic streams may be of similar pH to many streams receiving AMD, conductivity and metal concentrations are usually very low. Dissolved aluminium readily combines with humic and fulvic acids, resulting in some streams with low pH and low concentrations of labile aluminium (Sparling and Lowe 1996). In contrast to streams receiving AMD, benthic invertebrates and fish can be abundant within these naturally acidic systems, often at a similar richness to circum-neutral streams (Winterbourn and Collier 1987, Collier et al. 1990, Greig et al. 2010, Hogsden and Harding 2012b). In one of the few studies on algae in naturally acidic New Zealand streams, Collier and Winterbourn (1990) found both diatoms typical of acidic (e.g. *Eunotia* species) and circum-neutral environments (e.g. *Fragilaria* species). The presence of species typical of circum-neutral waters in streams of low pH may indicate adaptation of algae to acidic environments, as has been suggested for benthic invertebrates and fish (Collier et al. 1990, O'Halloran et al. 2008, Greig et al. 2010).

Streams impacted by AMD are typically monitored for changes in water chemistry and benthic invertebrate community composition (Pope et al. 2005). However, in severely impacted streams, benthic invertebrate communities are often depauperate, making robust statistical analyses difficult (Scullion and Edwards 1980, Simmons et al. 2005). In contrast, algae often proliferate in even the most severely impacted streams (e.g. Bray et al. 2008). Diatoms have been shown to respond predictably to a variety of stressors associated with AMD, including pH (Battarbee et al. 2010), elevated heavy metal concentrations (Hill et al. 2000, Ferreira da Silva et al. 2009), and metal precipitates (McKnight and Feder 1984). Several studies in the United States have shown that diatoms respond in a predictable manner along a gradient of acid mine drainage (Verb and Vis 2000, Hamsher et al. 2004, Smucker and Vis 2009, Zalack et al. 2010). Most diatoms are pH specialists (Telford et al. 2006) and pH has been shown to be an overriding factor in determining diatom community composition within AMD-contaminated streams (Verb and Vis 2000, Bray et al. 2008). Despite the abundance and potential indicator value of diatoms in streams receiving AMD, they are not currently used in monitoring this impact within New Zealand.

Previous studies assessing diatoms as indicators of pH have typically focused on either naturally or anthropogenically acidic streams, but rarely do they occur in the same region. As a result, of the studies in the literature that have trialled the use of diatoms as indicators of AMD I have not found any that have included naturally acidic streams (Verb and Vis 2000, de la Peña and Barreiro 2009, Smucker and Vis 2009, Zalack et al. 2010). The aim of this study was to determine the response of diatom communities to a gradient of acid mine drainage, including both naturally circum-neutral and acidic streams.

3.2 Methods

3.2.1. Survey sites

Thirty-nine streams ranging from 1st to 3rd order in the Buller and Grey Districts, West Coast, South Island, New Zealand were chosen to represent a range of pH conditions from circum-neutral reference (i.e. non-AMD) streams to those severely impacted by AMD (Fig. 3.1). All streams were located within a single ecoregion – the Westland Forest (Harding et al. 1997) and had similar climate, geology and vegetation. Each stream was sampled on a single occasion during baseflow conditions between January and April 2011 (austral summer and fall).

3.2.2. Physicochemical variables

Physical measurement protocols followed Harding et al. (2009). At each stream a reach representing 10x the wetted-width was established. Spot measurements of specific conductivity, pH, dissolved oxygen, and water temperature were taken using handheld meters (YSI550A and YSI63; YSI Incorporated, Yellow Springs, Ohio) and depth and width were recorded at four and three randomly selected locations along each reach, respectively. Current velocity was measured at four locations with a Flo-Mate 2000 (Marsh-McBirney, Frederick, Maryland). Canopy shading was estimated at three locations using a convex densiometer held at waist height, mid-channel in the upstream, midstream, and downstream sections of the reach. The streambed substrate composition was visually estimated using the Wolman Walk (Wolman 1954), in which 30 substrate particles were randomly selected and assigned to Wentworth particle classes. A Substrate Index was then calculated for each stream following Jowett and Richardson (1990). Iron hydroxide precipitate was visually quantified by estimating percentage cover in a 1-m^2 quadrat placed on the streambed at three random locations along the reach. Stream water was collected in acid washed containers, filtered, and kept on ice for later analysis of dissolved metals, nutrients, and dissolved organic carbon (DOC).

Laboratory analyses of water samples followed standard protocols (APHA 1995). Water samples for metal analysis were preserved with ultra pure HNO_3 and analysed by inductively coupled plasma mass spectrometry (ICP MS) for dissolved Al, Fe, Mn and Zn. Nutrients (NO_3^- and PO_4^{3-}) were analysed using an Easychem Plus (Systea, Anagni, Italy), an automated colorimetric analyser. DOC samples were preserved with HPO_4 and analysed using an Apollo 9000 Total Organic Carbon Combustion Analyzer (Teledyne Instruments, Mason, Ohio). All analyses were peformed at the University of Canterbury.

3.2.3. Diatom sample collection

In an initial survey, epilithic diatoms were collected by sampling 10 cobbles throughout the study reach at each of the 39 streams. The selection of cobbles was representative rather than random; where possible, pools and areas of heavy shade were avoided (Kelly et al. 1998). Preliminary taxonomic analysis on the epilithic sample revealed very low diatom cell densities. Thus, epiphytic diatoms were also sampled at each site by collecting visible filamentous algae and moss in all available benthic habitats throughout the study reach. Moss and algae were gently removed from the substrate with a stiff-bristled toothbrush and combined with stream water into a single 150 mL subsample. Samples were preserved with Lugol's iodine and stored at 4°C until analysis. The epiphytic sample was used for all taxonomic analysis.

3.2.4. Diatom sample preparation and identification

The diatom sample was vigorously shaken and a 5 mL sub-sample of the algal suspension as well as a small clipping of plant material was acid cleaned using concentrated H_2SO_4 and 30% H_2O_2 following the methods described by Biggs and Kilroy (2000). Diatoms were rinsed with distilled water 8 – 10 times with a minimum seven-hour settling period between rinses. A sub-sample of cleaned diatoms were then transferred to a coverglass and

allowed to air dry. Permanent slides were created using Naphrax, a mounting medium of high refractive index (refractive index: 1.73, Brunel Microscopes Ltd, Wiltshire, UK).
A total of 400 diatom valves per site were identified to the lowest possible taxonomic level (typically species) by transect scanning at 1000x magnification under oil immersion. Species were identified primarily using the taxonomic keys of Krammer and Lange-Bertalot (1991a, b, 1997, 2008) as well as Patrick and Reimer (1966, 1975), Krammer (2000) and Biggs and Kilroy (2000). Where necessary, taxonomy was updated using the online database AlgaeBase (Guiry and Guiry 2012). Taxa were categorised as acidophilic (optimum pH between 5.5 and 7.0) or acidobiontic (optimum pH < 5.5) using the ecological indicator values of van Dam et al. (1994). Acidophilic and acidobiontic taxa were combined into a single group of acid-tolerant taxa.

3.2.5. Statistical analyses

Taxonomic richness and Pielou's evenness (Pielou 1966) were calculated for each stream. Taxonomic richness was first plotted against pH and log(conductivity). These results clearly showed a two-phase response, with a drastic decrease in richness at low pH and high conductivity. Breakpoint regressions were selected to model the response of taxonomic richness against pH and conductivity and identify where breakpoints occur (Muggeo 2003). Breakpoint regressions were performed in the statistical package R (Version 2.13.0, R Development Core Team, Vienna, Austria) and linear regressions between Pielou's evenness and water chemistry variables were performed in SigmaPlot (Version 11.0, Systat Software Inc, San Jose, California).

Environmental variables were assessed for normality (Shapiro-Wilk test, $p > 0.05$) and where necessary normalised to meet assumptions. Streams were categorised into varying levels of AMD impact using a hierarchical cluster analysis (Euclidean distance measure, Ward's linkage method) based on water chemistry and physical variables typically associated with AMD: pH, conductivity, percentage iron hydroxide cover, and heavy metal concentrations (Al and Fe). Reference streams were categorised as naturally acidic at pH 5.7. Studies in the UK have suggested pH 5.7 as a threshold at which there is a distinct shift in the composition of aquatic biota (Sutcliffe and Carrick 1973, Sutcliffe and Hildrew

1989). In New Zealand, Collier and Winterbourn (1987) and Harding and Boothroyd (2004) suggested naturally acidic streams tend to occur in pH 4.3 – 5.7.

Multivariate analysis of variance (MANOVA) and Tukey's HSD tests were used to determine if there were significant differences in physical and chemical parameters between stream categories and where those differences occurred. A Detrended Correspondence Analysis (DCA) on square-root transformed relative abundance data and a MANOVA on axis 1, 2 and 3 site coordinates from the DCA were then performed to determine if diatom community composition differed among stream categories. The DCA gradient length of the first axis was greater than four standard deviations (4.63), indicating a strong unimodal response. Thus, constrained ordination techniques such as Canonical Correspondence Analysis (CCA) are appropriate to explore species-environmental relationships (ter Braak and Verdonschot 1995). To prevent extremely rare taxa from disproportionately influencing the analysis, taxa were only included in the CCA if they were found in more than one stream and with a relative abundance > 1% in at least one stream (Smucker and Vis 2009). A CCA was first run with all environmental variables. Forward selection using a Monte-Carlo permutation test (499 permutations, Hill's scaling) indicated which variables significantly ($p < 0.05$) contributed to the model. The CCA was then re-run using only significant environmental variables. DOC was not selected in the stepwise procedure and was included as a supplementary (i.e. passive) variable. DOC is important in streams receiving AMD as it decreases the bioavailability of heavy metals (Stokes et al. 1985). Supplementary variables are included in the ordination diagram as a visual aid but do not influence the ordination axes. All ordinations were performed using Canoco (Version 4.5, Microcomputer Power, Ithaca, New York). MANOVAs, Tukey's HSD tests and correlations were performed in the statistical package R.

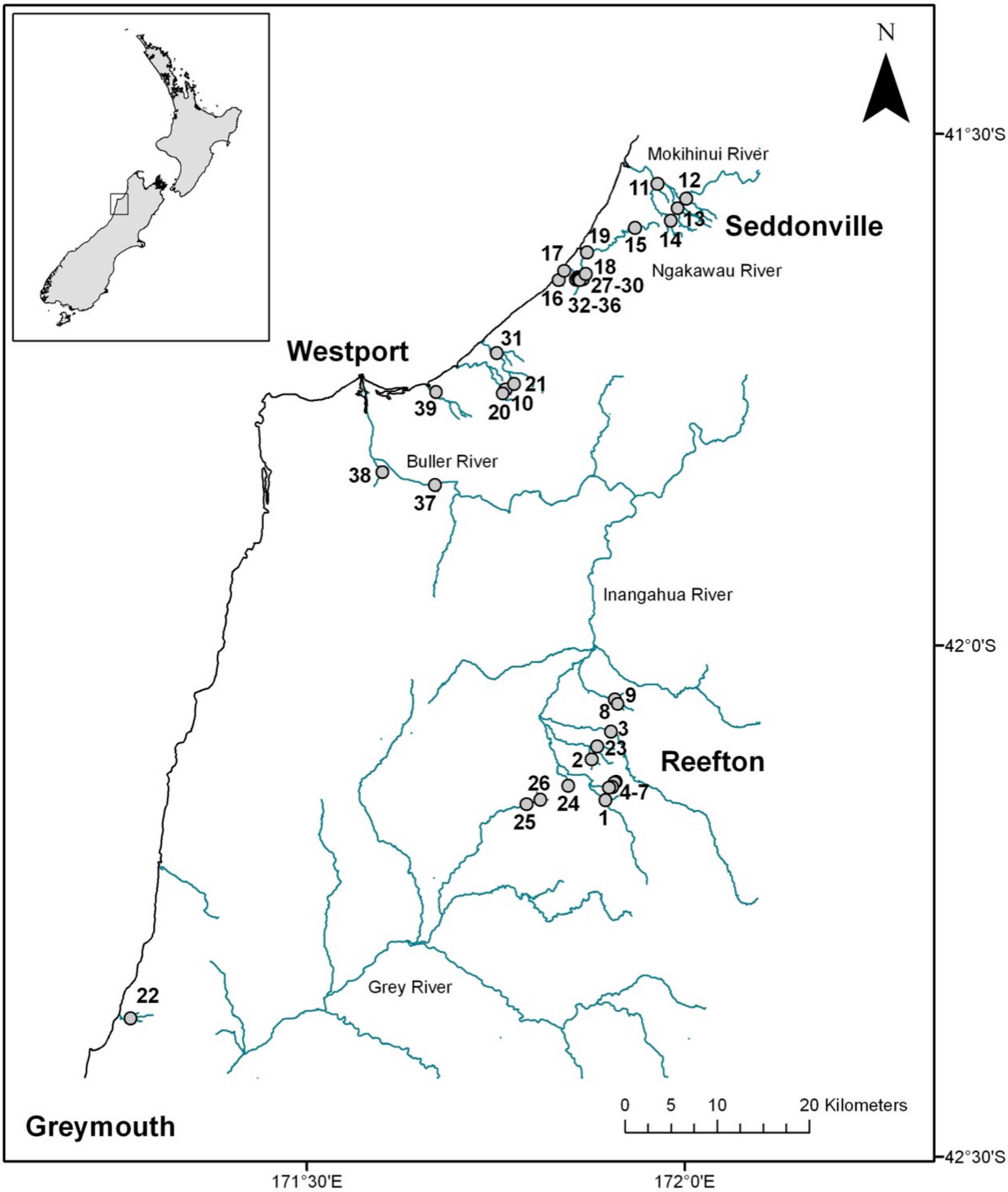


Figure 3.1. Streams surveyed for water chemistry, physical conditions and algae (n = 39) on the West Coast, South Island, New Zealand from January through April 2011.

3.3. Results

3.3.1. Stream characteristics and categorisation

There was a wide range in water chemistry across the 39 streams (pH: 2.1 – 7.7, specific conductivity: 22 – 1,919 μS_{25} /cm, Al: 0.02 – 59.16 mg/L, Fe: 0.01 – 18.91 mg/L, Zn: 0.01 – 2.2 mg/L, Mn: 0 – 2.06 mg/L). Iron hydroxide precipitates were present at 13 streams (range 7 – 100%) and covered 100% of the streambed at four streams. Conductivity was strongly correlated with Al ($r = 0.92$, $p < 0.0001$), Fe ($r = 0.76$, $p < 0.0001$), Zn ($r = 0.98$, $p < 0.0001$) and Mn ($r = 0.98$, $p < 0.0001$).

Streams were separated into four AMD impact categories based on results of the hierarchical cluster analysis as well as the pH threshold established for naturally acidic streams: circum-neutral reference, naturally acidic reference, moderately and severely impacted by AMD. One stream (Stream #20) was a borderline classification. This stream was sampled below the confluence of a severely impacted abandoned adit and the circum-neutral headwaters. This stream was assigned to the moderately impacted category. An additional stream, Stream #3, was naturally acidic but flowed near abandoned coal works. Based on results of the cluster analysis, this stream was classified as naturally acidic at the time of sampling.

Physicochemical parameters were significantly different between the four AMD impact categories (MANOVA, $F_{df=32} = 23.76$, $p < 0.0001$). Severely impacted streams differed significantly from each of the other three stream categories in conductivity, pH and heavy metal concentrations (Table 3.1). However, conductivity and metal concentrations were not significantly different between reference (both naturally acidic and circum-neutral) and moderately impacted streams (Table 3.1). Moderately impacted streams had a wide range in both conductivity (43 – 313 μS_{25}/cm) and Al concentrations (0.07 – 9.98 mg/L). Iron hydroxide was present in all moderately impacted streams, indicating fluctuating water chemistry, which may not be captured by spot measurements. Physical variables (e.g. canopy shading, velocity, substrate) did not differ between the four categories (Table 3.1).

Table 3.1. Means (± SEM) and univariate ANOVA statistics of physicochemical variables in circum-neutral reference (Ref. C), naturally acidic reference (Ref. NA), moderately and severely AMD-impacted streams. NS = not significant (p > 0.05). Significant differences (p < 0.05) using Tukey's HSD are indicated by different superscript letters.

Variable	Ref. C $n = 16$	Ref. NA $n = 4$	Moderate $n = 8$	Severe $n = 11$	F	P
pH	6.5^{a} (±0.13)	4.6^{b} (±0.39)	4.2^{b} (±0.31)	2.8^{c} (±0.14)	202.7	< 0.0001
Conductivity (μS_{25}/cm)	63^{a} (±10)	32^{a} (±7)	160^{a} (±38)	1363^{b} (±109)	58.1	< 0.0001
Al (mg/L)	0.1^{a} (±0.02)	0.6^{a} (±0.39)	2.3^{a} (±1.19)	36.2^{b} (±2.87)	60.49	< 0.0001
Fe (mg/L)	0.2^{a} (±0.06)	0.2^{a} (±0.05)	3.0^{a} (±2.29)	9.8^{b} (±0.59)	43.24	< 0.0001
DOC (mg/L)	3.07^{a} (±0.43)	6.17^{b} (±2.17)	2.12^{a} (±0.53)	1.62^{a} (±0.35)	5.76	0.022
Iron hydroxide (%)	0^{a} (±0)	0^{a} (±0)	90^{b} (±4.8)	16^{c} (±7.3)	5.26	0.029
NO_3^- (mg/L)	0.08^{a} (±0.02)	0.18^{a} (±0.15)	0.10^{a} (±0.04)	0.91^{a} (±0.45)	4.35	0.045
PO_4^{3-} (mg/L)	0.27^{a} (±0.10)	0.68^{a} (±0.60)	0.02^{a} (±0.01)	0.33^{a} (±0.09)	0.12	NS
Temperature (°C)	14.1^{a} (±0.75)	14.8^{a} (±0.81)	12.6^{a} (±0.59)	16.5^{b} (±0.24)	2.61	NS
DO (mg/L)	9.5^{a} (±0.64)	8.8^{a} (±0.3)	9.6^{a} (±0.34)	9.7^{a} (±0.24)	0.13	NS
Current velocity (m/s)	0.23^{a} (±0.03)	0.14^{a} (±0.03)	0.19^{a} (±0.07)	0.18^{a} (±0.02)	0.99	NS
Canopy cover (%)	52^{a} (±10)	52^{a} (±27)	58^{a} (±9.83)	63^{a} (±9.11)	0.90	NS
Depth (m)	0.17^{a} (±0.02)	0.20^{a} (±0.06)	0.14^{a} (±0.04)	0.16^{a} (±0.01)	0.89	NS
Width (m)	4.2^{a} (±0.76)	1.7^{a} (±0.49)	2.4^{a} (±0.61)	3.1^{a} (±0.83)	1.74	NS
Substrate Index	5.8^{a} (±0.11)	5.7^{a} (±0.25)	6.0^{a} (±0.35)	5.5^{a} (±0.33)	0.60	NS

Note: due to missing data, velocity measurements for Ref. NA and moderate AMD sites are n = 3 and n = 7, respectively and the NO_3^- Ref. C and Ref. NA measurements are n = 14 and n = 3, respectively.

3.3.2. Diversity and abundance

A total of 113 diatom taxa in 39 genera were identified (Appendix B). Six taxa were unable to be identified to generic level. Many species were rare: 54% of taxa were found at two or fewer streams and 19% were found as a single valve at a single stream. The commonest were *Karayevia oblongella* (Østrup) M. Aboal (27/39 sites) and *Gomphonema parvulum* Kützing (Kützing) (24/39 sites). The mean relative abundance of *K. oblongella* and *G. parvulum* was 10.3% (range 0.25% – 54.5%) and 7.6% (range 0.25% – 51.5%) respectively.

There was a wide range in diatom richness observed in reference (both circum-neutral and naturally acidic) as well as moderately impacted streams (Fig 3.2A, B). Mean taxonomic richness in circum-neutral and naturally acidic streams was 19.4 and 15.8 respectively; however, moderately impacted streams showed the greatest range in taxonomic richness, from 8 to 33 taxa per stream (mean 20.8 taxa). Taxonomic richness decreased markedly at a threshold of pH 3.35 (95% CI; 3.2 – 3.4) (Fig. 3.2A). The number of taxa below this threshold ranged from 1 – 5 (mean 2.7 taxa). The threshold for specific conductivity was estimated to be between 313 and 931 μS_{25}/cm (Fig. 3.2B). As there were no streams sampled with a specific conductivity within this range, a more specific threshold for this parameter could not be established.

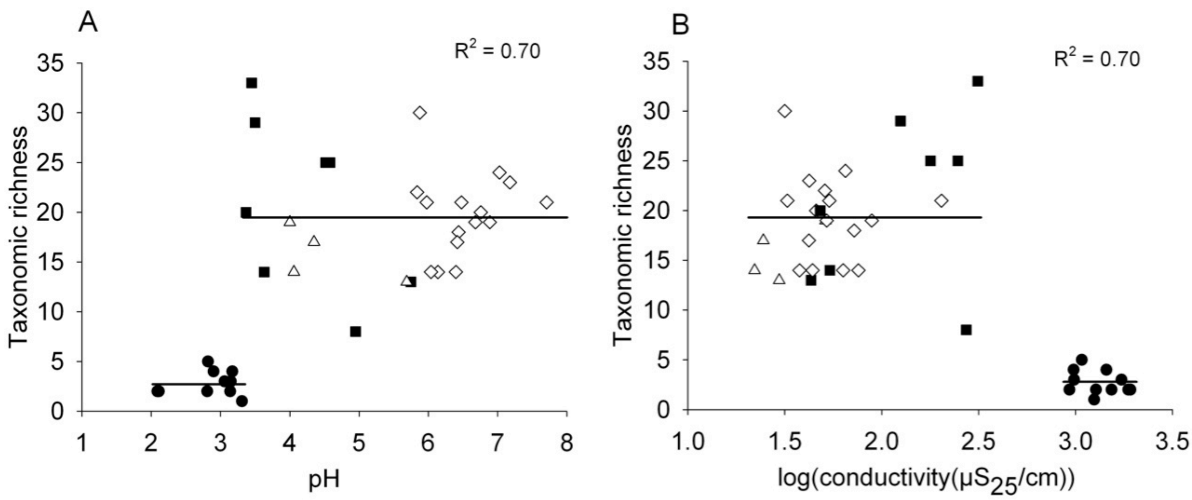


Figure 3.2. Breakpoint regression analysis of taxonomic richness versus pH (A) and conductivity (B). Circle = severe AMD, square = moderate AMD, diamond = circum-neutral reference, and triangle = naturally acidic reference.

In contrast to taxonomic richness, evenness generally declined linearly with decreasing pH and increasing conductivity, although severely impacted streams had a wide range in evenness values (Fig. 3.3A, B). For example, two species were identified at a severely impacted stream, but in relatively high evenness: 31% *Nitzschia paleaeformis* Hustedt and 69% *Pinnularia* cf. *acidophila* Hoffman and K. Krammer. Neither evenness nor taxonomic richness was correlated with percentage iron hydroxide cover.

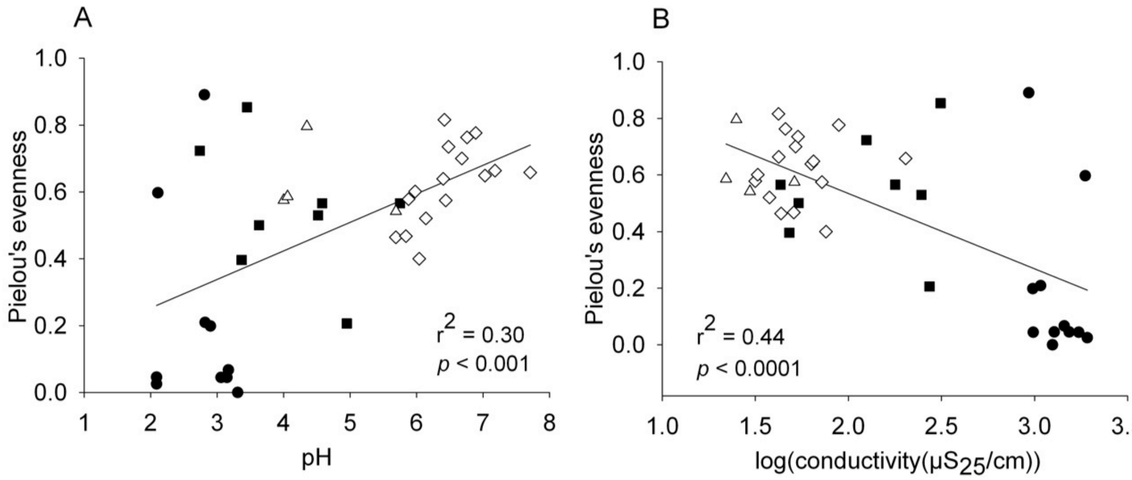


Fig. 3.3. Pielou's evenness versus pH (A) and conductivity (B). Circle = severe AMD, square = moderate AMD, diamond = circum-neutral reference, and triangle = naturally acidic reference.

3.3.3. Acid-tolerance

The relative abundance of acid-tolerant taxa decreased as pH increased (r = -0.85, p < 0.0001). Acid-tolerant taxa included species of *Eunotia*, *Frustulia*, *Navicula*, *Nitzschia*, *Brachysira*, *Tabellaria*, and *Pinnularia*. These taxa were found in streams with a wide range of pH values. However, when found in circum-neutral streams their abundances were often very low (one or two valves per stream). With the exception of *Tabellaria flocculosa* (Roth) Kützing, all acid-tolerant species were found at their maximum abundance in streams with pH ≤ 5.0.

3.3.4. Community composition

A DCA of community composition clearly separated streams into three groups along Axis 1 (Fig. 3.4) and a MANOVA indicated significant differences in diatom community composition between the four stream categories largely associated with Axis 1 (Axis 1: $F_{df=3} = 288.53$, $p < 0.0001$, Axis 2: $F_{df=3} = 3.24$, $p = 0.03$). There was no statistically significant difference in community composition between naturally acidic and moderately impacted streams (Tukey HSD of Axis 1 values, $p = 0.9479$). *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst was dominant in streams plotted near the top of Axis 2, and species characteristic of streams plotted near the bottom of Axis 2 include *Eunotia bilunaris* (Ehrenberg) Schaarschmidt, *Eunotia minor* (Kützing) Grunow, *Frustulia crassinervia* (Brébisson) Lange-Bertalot & Krammer and *Frustulia saxonica* Rabenhorst.

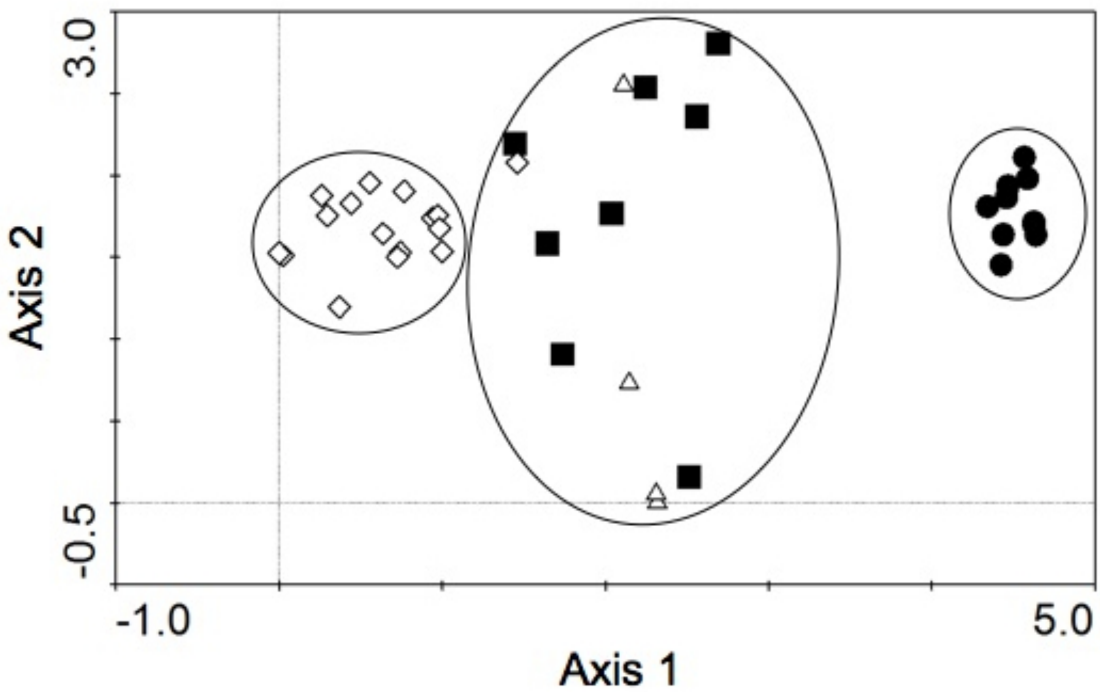


Figure 3.4. DCA biplot of diatom community composition at the 39 streams. The percentage variance explained by Axis 1 and 2 was 16.1% and 22.7%, respectively. Circle = severe AMD, square = moderate AMD, diamond = circum-neutral reference, and triangle = naturally acidic reference.

Circum-neutral reference streams were characterised primarily by *Karayevia*, *Gomphonema*, *Rossithidium*, *Cocconeis*, and *Planothidium* species (Fig. 3.5). The average relative abundance of genera was similar in naturally acidic and moderately impacted streams, with *Eunotia* as the dominant genus in both stream categories (Fig. 3.5). Severely impacted streams were dominated by *Pinnularia*, with *P.* cf. *acidophila* at 69 – 100% relative abundance.

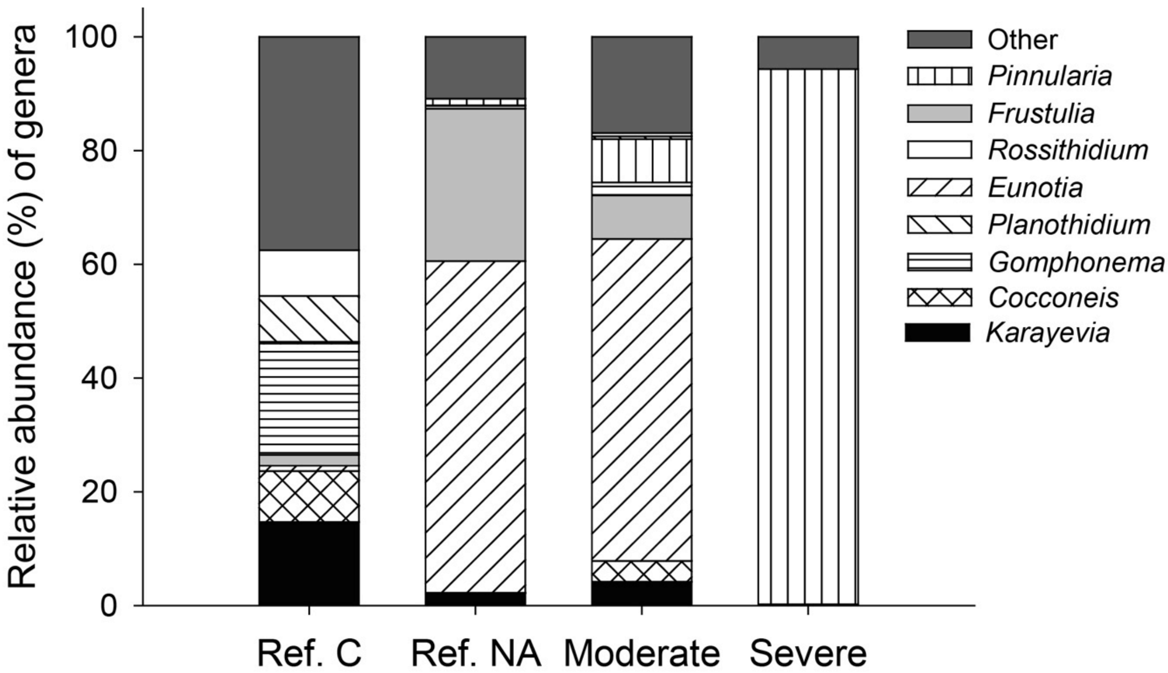


Figure 3.5. Average relative abundance (%) of diatom genera in severe AMD, moderate AMD, naturally acidic reference (Ref. NA) and circum-neutral reference (Ref. C) streams. "Other" genera include six taxa unable to be identified to generic level as well as those found in ≤ 7% relative abundance in all four categories.

3.3.5. Relationships between species and environmental parameters

CCA indicated that community composition was significantly correlated with pH, Al and iron hydroxide deposits ($r = 0.961$ and $r = 0.857$ for Axis 1 and 2, respectively). Axis 1 was driven by an aluminium and pH gradient, with streams with positive Axis 1 scores representing moderately to severely impacted streams of high aluminium concentrations and low pH (Fig. 3.6). Community composition along Axis 2 was driven by pH and to a lesser extent iron hydroxide deposition. Streams with positive Axis 2 scores included naturally acidic reference streams and streams moderately impacted by AMD (Fig. 3.6).

Although naturally acidic reference streams did not have iron hydroxides present, the communities at these streams were similar to moderately impacted streams with metal oxide deposition.

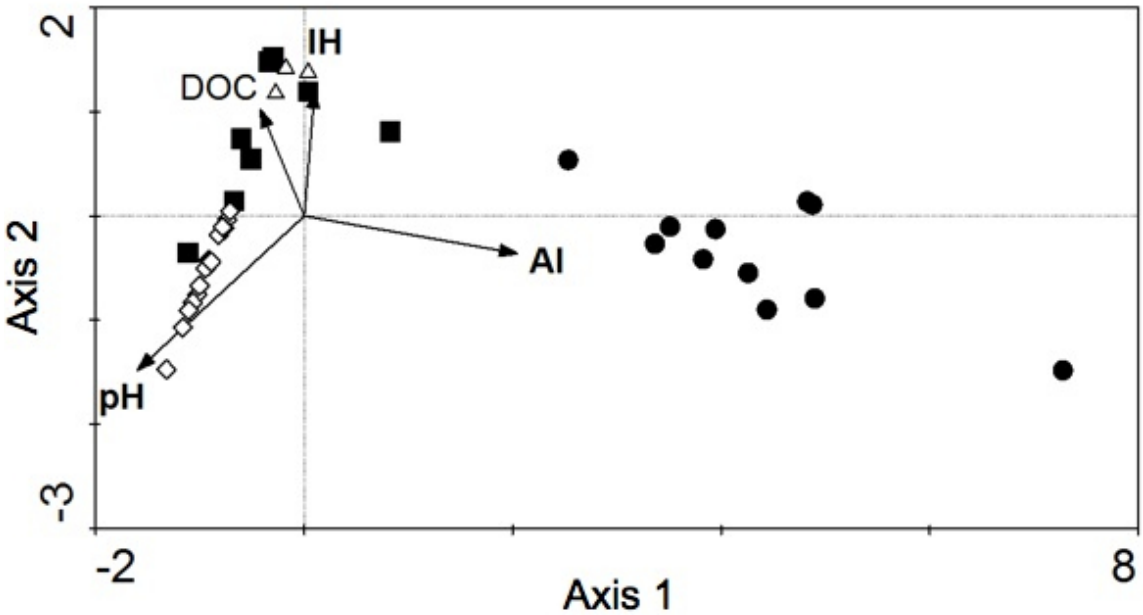


Figure 3.6. CCA biplot of diatom community composition at the 39 streams. Iron hydroxide (IH), aluminium, and pH were identified as significant ($p < 0.05$) environmental variables in structuring diatom community composition. DOC was included as a supplementary variable. Circle = severe AMD, square = moderate AMD, diamond = circum-neutral reference, and triangle = naturally acidic reference.

3.4. Discussion

Diatom diversity and community composition differed markedly between circum-neutral reference, moderately and severely impacted streams. However, similar communities were found in non-impacted naturally acidic streams and streams receiving moderate levels of AMD. Naturally acidic streams were of similar pH to those moderately impacted by AMD, and there was no statistically significant difference in conductivity, Al or Fe concentrations between the two categories. Several moderately impacted streams did have higher conductivity and heavy metal concentrations than naturally acidic streams, and yet community composition was similar to naturally acidic streams with lower conductivity and metal concentrations, but of similar pH. These results indicate that diatom communities were strongly structured by pH and were able to tolerate moderate conductivities and concentrations of heavy metals without a shift in community composition.

The communities in both naturally acidic and moderate AMD streams were characterised by a high abundance of *Eunotia* species. *Eunotia* is primarily an acid-tolerant genus that is typical of streams impacted by AMD (Verb and Vis 2000, Niyogi et al. 2002). *Eunotia* species are characteristic of naturally acidic environments and dominate unpolluted humic streams of pH < 5.5 in Sweden (Andrén and Jarlman 2008, Cantonati and Lange-Bertalot 2011). The relative abundance of acid-tolerant species has been used as an indicator of AMD in the United States (Hamsher et al. 2004, Zalack et al. 2010). However, on the West Coast of New Zealand, a high abundance of acid-tolerant taxa such as *Eunotia* and *Frustulia* does not necessarily indicate the presence of AMD.

On the West Coast of the South Island, naturally acidic, naturally circum-neutral, and AMD streams can occur in close proximity. A similar configuration can be found in Sweden, in which streams receiving AMD from metal mining may be in the same region as naturally acidic streams (Malmqvist and Hoffsten 1999). However, I can find no examples in the literature on algae in AMD impacted streams in Sweden. In regions with several studies investigating algae in AMD streams (e.g. the United States), naturally acidic streams do not seem to occur in close proximity to mines. Landscape configuration and associated evolutionary history such as acid tolerance can complicate the use of diatoms in biomonitoring activities. More work needs to be done on identifying diatom species that might distinguish between naturally and anthropogenically acidified streams. For example, *Frustulia saxonica* prefers streams rich in humic acids, with reduced abundance in anthropogenically acidified streams (Lange-Bertalot 2001). In the present study, *F. saxonica* was found in both naturally acidic and moderate AMD streams, with its highest relative abundance in two naturally acidic streams (21 and 12.5%).

In streams severely impacted by AMD (pH ≤ 3.3 and specific conductivity ≥ 931 μS_{25}/cm), low pH and high concentrations of heavy metals, notably aluminium, filtered the diatom community towards a single acidobiontic species, *Pinnularia* cf. *acidophila*. The pH of severely impacted streams in the present study was well below levels commonly reported in other AMD algal studies (de la Peña and Barreiro 2009, Smucker and Vis 2009, Zalack et al. 2010). *Nitzschia paleaeformis*, another acid-tolerant species, was also common in severe AMD streams, at a relative abundance up to 31%. *P. acidophila* is a recently described species that was first identified in a lake resulting from open cast mining in Germany (Krammer 2000) and has been recorded in high abundance in streams receiving AMD in Korea (Kim et al. 2008). However, *Pinnularia* species found in mine seeps are notoriously difficult to identify (B. Van de Vijver, National Botanic Garden of Belgium, personal communication). *P. acidophila* is similar in appearance to *Pinnularia acoricola* Hustedt, and it is possible that *P. acidophila* has been occasionally misidentified as *P. acoricola.* The latter has a pH optimum near 2.3 and has been found in New Zealand hot springs with pH < 1 (Cassie and Cooper 1989, Owen et al. 2008). It also tolerates high heavy metal concentrations (Denys 1984) and has been recorded in high abundance in AMD-contaminated streams in Spain (de la Peña and Barreiro 2009, Urrea-Clos and Sabater 2009), Portugal (Luís et al. 2011), and the UK (Hargreaves et al. 1975).

My results are consistent with a review of diatoms in highly acidic environments (DeNicola 2000) that suggested many diatom species are unable to persist below pH 3.5. In the present study, taxonomic richness decreased rapidly at a threshold of pH 3.4, below which *P.* cf. *acidophila* was found in 69 – 100% relative abundance. These results are similar to those of Luís et al. (2009), who found at pH 1.5 – 3.5 in Portuguese AMD streams, there was low taxonomic richness dominated by *P. acoricola* at 87 – 91% abundance. *P.* cf. *acidophila* has the potential to act as an indicator of New Zealand streams severely impacted by AMD.

In Buller/Grey District streams, taxonomic richness increased in moderately impacted streams due to *P.* cf. *acidophila* being replaced by a diverse community of acid-tolerant species. Species typical of streams receiving moderate levels of AMD included *E. exigua*, *F. crassinervia* and *Brachysira brebissonii* R. Ross and these species have been commonly reported in AMD streams elsewhere (Douglas et al. 1998, DeNicola 2000 and references within, Verb and Vis 2000, Zalack et al. 2010). Iron hydroxide deposits were present in varying levels of intensity at all moderately impacted streams. However, there was no relationship between taxonomic richness and the percentage cover of iron hydroxide deposition at a stream. Previous studies on the effect of metal oxide deposition on in-stream flora and fauna have shown contrasting results. DeNicola and Stapleton (2002) found that metal precipitates did not significantly impact periphyton diversity or composition. In contrast, McKnight and Feder (1984) found intact diatom cells were absent from artificial substrate in the presence of metal precipitates, and concluded that metal precipitates significantly limited diatom colonisation and growth. The present study focused on epiphytic diatoms, which may be less impacted by iron hydroxide than epilithic diatoms due to the accumulation of precipitate on rocks.

Despite the challenge of using diatoms to differentiate between naturally acidic streams and streams moderately impacted by AMD, communities were markedly different between circum-neutral reference and both moderately and severely impacted streams. Therefore, diatoms have the potential to significantly contribute to AMD monitoring in regions where AMD and natural acidity occur. Diatoms may be especially useful in both monitoring restoration of an AMD-impacted stream where the overall goal is to restore a stream to low conductivity, circum-neutral conditions and in identifying when a stream has crossed a threshold from moderately to severely impacted by AMD.

Chapter 4

Development of a diatom-based biotic index and multimetric index for assessing AMD impacts in New Zealand streams

4.1. Introduction

Freshwater ecosystems are among the most threatened environments worldwide (Dudgeon et al. 2006). Globally an estimated 65% of freshwater habitats are considered moderately to severely threatened (Vörösmarty et al. 2010). Pressures on the remaining ecosystems continue to rise, particularly as demands for freshwater and human populations grow. Mining is one such activity that frequently results in long-term and widespread impacts on freshwater ecosystems (Palmer et al. 2010).

Historically, a number of different assessment tools have been used to assess the impacts of human activities on aquatic communities (Chapman 1996). Among the most commonly used have been biotic indices. The simplest indices comprise a single metric, which is used to indicate a degree of impact. Some biotic indices rely on measures of community structure (e.g. taxonomic richness) while others rely on calculation of tolerance values for differing taxa (e.g. MCI, the Macroinvertebrate Community Index, Stark 1993). More complex approaches use multimetric indices (e.g. IBI, the Index of Biotic Integrity, Karr 1981), the aim of which is to integrate several attributes (metrics) of a community that respond predictably to environmental change into a qualitative score which can then be used to assess the health of an ecosystem (Hering et al. 2006). Combining complex data on the structure and function of a community into a single score allows for easy communication to the public and decision makers regarding the status of freshwater ecosystems (Karr 1999). However, by combining data information is simplified and lost whereas other biomonitoring approaches attempt to maintain information. One such approach is predictive modelling (e.g. RIVPACS, the River InVertebrate Prediction and

Classification System, Wright 1995). This approach uses multivariate data by developing a model based on communities found in un-impacted reference streams. Stream health is then quantified by comparing the aquatic community at a test site to the community that is predicted to inhabit similar un-impacted environments (Norris and Hawkins 2000). Predictive models require a large number of reference sites to identify the range of species to be potentially found in un-impacted streams (Feio et al. 2007).

In New Zealand, stream health indices have been developed and tested with benthic invertebrates (Stark 1993, 1998, Wright-Stow and Winterbourn 2003, Gray and Harding in press) and predictive models with fish (Joy and Death 2004), but none have yet been developed for algae. Biological monitoring and pollution assessment of algae in New Zealand streams often focuses on non-taxonomic metrics, such as biomass (Welch et al. 1992) and percentage cover (Biggs 2000), rather than taxonomic features such as species richness and composition. This is despite the potential of algae to act as indicator species.

Diatoms in particular have several advantages as bioindicators and are widely used in monitoring programs (European Parliament Directive 2000/60/EC 2000, USEPA 2002, Lavoie et al. 2009, Stevenson et al. 2010). For example, diatoms are a species-rich group that can be found in almost all illuminated aquatic habitats; Mann and Droop (1996) estimate there are at least 200,000 diatom species worldwide. From a biomonitoring perspective diatoms may also be identified to species level or lower (i.e. variety) and most taxa have well-known ecological tolerances (Bahls 1993, van Dam et al. 1994). Diatoms also have a short generation time, days compared to months as in macroinvertebrates (Zalack et al. 2010). Therefore, they have the advantage of being able to integrate temporal changes to the environment (unlike spot water chemistry measurements), but respond more quickly to environmental changes than benthic invertebrates.

Diatom-based metrics have been used to assess a variety of stressors such as eutrophication (Kelly and Whitton 1995, Rott et al. 1998), acidification (Round 1990) and anthropogenic climate change (Mackay et al. 1998). More recently, the ability of diatoms to detect and monitor the impacts of acid mine drainage (AMD) has been investigated (Hamsher et al. 2004, Smucker and Vis 2009, Zalack et al. 2010). AMD is generated when sulphide-bearing coal reacts with air and water, producing sulphuric acid and releasing associated heavy metals. AMD may contaminate both surface and groundwater, resulting in streams of low pH and elevated metal concentrations (Akcil and Koldas 2006). Diatom communities have been shown to respond in a predictable manner across an AMD gradient at a variety of spatial scales (Verb and Vis 2000, de la Peña and Barreiro 2009, Smucker and Vis 2011a). In the Western Allegheny Plateau, Ohio, indices of biotic integrity based on diatoms (the AMD-Diatom Index of Biotic Integrity, AMD-DIBI, and the Diatom Model Affinity Index, DMA) as well as periphyton including diatoms (the Periphyton Index of Biotic Integrity, PIBI) were able to successfully categorise streams according to the level of AMD impairment (Hamsher et al. 2004, Smucker and Vis 2009, Zalack et al. 2010).

While researchers in the United States have had success in developing diatom-based indices, this does not necessarily imply that they can be applied in other regions of the world. Indices may respond differently within different ecoregions, and ideally should be developed for the region in which they are to be used (Pipp 2002, Potapova and Charles 2007, Danielson et al. 2011). A significant number of freshwater ecosystems in the Buller and Grey Districts in New Zealand are impacted by AMD and no attempts have been made to test the usefulness of diatoms as indicators of mining impacts in these regions. The aim of this study was to develop a diatom-based index for New Zealand streams receiving AMD. Initially, two indices, a single biotic index and a multimetric index, were developed and tested. The two indices were then compared to determine the index that best identified AMD impacts in New Zealand streams.

4.2. Methods

4.2.1. Study sites

Thirty-nine streams were selected to represent a gradient from non-impacted to severe AMD on the West Coast of the South Island, New Zealand. At each stream a reach approximately 10x the wetted width was selected. All streams were sampled on a single occasion during baseflow conditions between January and April 2011. They were located within a single ecoregion, the Westland Forest (Harding et al. 1997), and were of similar climate, geology and vegetation. The region includes several areas of extensive historic and active coal mining as well as regions of native forest with naturally acidic, brown water streams of pH as low as 4. This natural acidity is primarily produced by humic and fulvic acids from organically enriched soil and swampy peatland (Winterbourn and Collier 1987).

4.2.2. Physicochemical parameters

Physicochemical assessment as well as diatom sample collection and enumeration are described in detail in Chapter 3. Measurements of pH and specific conductivity (μS_{25}/cm) were taken at each site using hand held meters (YIS Incorporated, Yellow Springs, Ohio). Water samples were also collected and filtered on site for later analysis of dissolved metals (Al, Fe, Mn and Zn) by inductively coupled plasma mass spectrometry (ICP-MS). Average percentage iron hydroxide deposition was quantified by placing a 1-m^2 quadrat at three random locations along the streambed and visually estimating the area within the quadrat covered by iron hydroxide.

At each stream, a 12.5-cm^2 scraping was collected from the uppermost surface of ten cobbles using a stiff-bristled brush, and the epilithic diatom slurry was combined into a single composite sample. Samples were filtered through 47 mm Whatman GF/C filters and analysed spectrophotometrically (Shimadzu UV-1601 PC, Japan) to determine chlorophyll *a*/m^2 as a measure of diatom biomass (Biggs and Kilroy 2000). Epiphytic diatoms were also sampled at each stream by collecting filamentous algae and moss. A 5 mL sub-sample of the epiphytic sample was acid cleaned and mounted in Naphrax (Brunel Microscopes Ltd. Wiltshire, UK). A total of 400 valves were identified per site to the lowest level possible (typically species) primarily using the taxonomic keys of Krammer and Lange-Bertalot (1991a, b, 1997, 2008).

4.2.3. Statistical analyses

One-way ANOVA and correlations were performed in the statistical package R (Version 2.13.0, R Development Core Team, Vienna, Austria), and ordinations in Canoco (Version

4.5, Microcomputer Power, Ithaca, New York). Environmental variables were normalised prior to analysis when necessary. A hierarchical cluster analysis on pH, conductivity, percentage iron hydroxide deposition, Al and Fe concentrations categorised streams into three groups: reference, moderately or severely impacted by AMD. Reference streams were further divided into two categories: circum-neutral reference (pH > 5.7) and naturally acidic reference (pH ≤ 5.7).

4.2.4. Single Biotic Index development

A single Biotic Index was developed following the weighted averaging methodology outlined by Smith et al. (2007) and Blakely and Harding (2010). A Canonical Correspondence Analysis (CCA) using forward selection (Mote Carlo Permutation test, 499 permutations) was performed on diatom relative abundance data and water chemistry parameters to determine the most important environmental variable in structuring diatom community composition at a stream. This variable was then used for Biotic Index development.

Diatom taxonomic lists were created for each stream. Taxa were only included in the analysis if they were present in > 3 streams. Streams were then sorted in ascending order according to the environmental variable identified in the CCA and divided into ten bins (4 streams in 9 bins and 3 streams in 1 bin). An environmental optimum for each taxon was estimated using the following equation (Smith et al. 2007):

$$\textit{Environmental optimum} = \frac{\sum (W_{prop})_{bin1+bin2...+bin10}}{\sum (U_{prop})_{bin1+bin2...+bin10}}$$

Where U_{prop} = taxon frequency in a bin x total taxon frequency in all bins and W_{prop} = the mean environmental variable of the bin x U_{prop}. The environmental optimum for each taxon was then ranked in ascending order and divided into 10 bins (4 taxa in 7 bins and 5 taxa in 3 bins). Taxa in the bin with the lowest optimal value were given a tolerance score of 1, and taxa in the bin with the highest optimal value were given a tolerance score of 10.

A quantitative Biotic Index score was then calculated for each stream using the following equation (Stark 1993):

$$\textit{Quantitative Biotic Index score} = \sum_{i=1}^{i=S} \frac{(n_i \times a_i)}{N}$$

Where S is the total number of diatom taxa observed at a site, n_i is the number of valves in taxon *i*, a_i is the tolerance score of taxon *i,* and N is the total number of valves counted at a site. Final index scores were rounded to the nearest whole number. When developing an index, quantitative data are preferable over presence-absence data (Round 1991, Stevenson and Bahls 1999). Diatom valves may drift into the study reach from a different environment, and the presence of a small number of valves does not necessarily reflect the environmental conditions of a site at the time of sampling.

4.2.5. Multimetric Index development

A second index based on multiple metrics was developed following the methods outlined by Wang et al. (2005) and Zalack et al. (2010). From a pool of potential metrics, four were selected based on correlations with water chemistry (pH and Al concentrations) and separation power of box plots. These were: acid tolerance, dominant genera, indicator species, and similarity to circum-neutral reference streams (Table 4.1). Acid-tolerant taxa were identified using the ecological tolerance values of van Dam et al. (1994). Their study lists the pH tolerance values of 948 taxa and is widely used in diatom-based bioassessment (Fore and Grafe 2002, Hill et al. 2003, de la Peña and Berreiro 2009). A common species on the West Coast of New Zealand, *Pinnularia* cf. *acidophila* Hofmann & K. Krammer, was first described in 2000 and is not listed in van Dam et al. (1994). This species has been found in highly acidic environments (Krammer 2000, Kim et al. 2008) and was categorised as acid-tolerant in the present study. Indicator species analysis and average Bray-Curtis similarity to circum-neutral reference streams were calculated in PC-ORD (Version 4.01, MjM Software, Gleneden Beach, Oregon). Indicator species were significantly ($p > 0.05$) more likely to occur in circum-neutral reference streams than each of the other AMD impact categories (Monte Carlo test of significance, 1,000 permutations).

Two metrics were predicted to increase with AMD impairment, and two metrics were predicted to decrease (Table 4.1). Metrics were normalised using a 0 – 10 scoring system (Wang et al. 2005). For metrics that increased with impairment, scores were calculated by dividing the metric value at a stream by the 90th percentile value and multiplying by 10. For metrics that decreased with impairment, scores were calculated by dividing the metric value at a stream by the 90th percentile value, subtracting this value by 1 and multiplying by 10. Ten was the maximum possible score for each metric. For each stream, the Multimetric Index was calculated by summing the four metric scores and rounding to the nearest whole number. Index scores may range from 0 (highly impaired) to 40.

Table 4.1. Four diatom metrics included in the Multimetric Index, their calculation and predicted response to impairment.

Attribute type	Metric	Metric calculation	Response to impairment
Autecology	Acid tolerance	% relative abundance of acidophilic and acidobiontic taxa	Increase
Diversity	Dominant genera	% relative abundance of the dominant genera	Increase
Community composition	Similarity to reference streams	Average Bray-Curtis similarity to circum-neutral reference streams	Decrease
Community composition	Indicator species	% relative abundance of species typical of circum-neutral reference streams	Decrease

4.3. Results

4.3.1. Diatom communities across the AMD gradient

A total of 113 diatom taxa in 39 genera were recorded across the 39-stream AMD gradient (pH: 2.1 – 7.7, specific conductivity: 22 – 1,919 μS_{25}/cm, Al: 0.02 – 59.16 mg/L, Fe: 0.01 – 18.91 mg/L, iron hydroxide deposition: 0 – 100%). Sixteen streams were classified as circum-neutral reference, four as naturally acidic reference, eight as moderately impacted and 11 as severely impacted (Appendix A). Taxonomic richness ranged from only one at a stream severely impacted by AMD to 33 at a moderately impacted stream. Severely impacted streams were dominated by *P.* cf. *acidophila* (relative abundance 69 – 100%) and *Nitzschia paleaeformis* Hustedt (relative abundance 0 – 31%), while *Eunotia* and *Frustulia* were the two most common genera in both naturally acidic and moderately impacted streams. In circum-neutral reference streams, the dominant genera were *Gomphonema*, *Planothidium*, *Rossithidum*, *Cocconeis*, and *Karayevia*.

4.3.2. Single Biotic Index

CCA results indicated that community composition was strongly driven by pH and aluminium concentrations. Aluminium and pH values were highly correlated (r = -0.78, $p < 0.0001$). Because pH values at the 39 streams spanned a pH gradient and there was a wide gap in aluminium concentrations between moderately (0.07 – 9.98 mg/L) and severely (21.14 – 59.16 mg/L) impacted streams, pH was selected as the environmental variable to develop the Biotic Index (the pHBI).

Not surprisingly the majority of diatom taxa (70 taxa) were found in ≤ 3 streams. In general, rare taxa were found in low relative abundance: 91% of taxa occurring in three or fewer streams were found in ≤ 5% relative abundance. Tolerance values were calculated for 43 taxa (Table 4.2). pHBI scores ranged from 1 – 9 and were strongly correlated with pH (r = 0.92, $p < 0.0001$), specific conductivity (r = -0.70, $p < 0.0001$), and Al concentrations (r = -0.71, $p < 0.0001$). pHBI scores differed significantly between stream categories (One-way ANOVA, $F_{df=3}$= 107.75, $p < 0.0001$). However, pHBI scores were not significantly different between naturally acidic reference and moderately impacted streams (ANOVA Tukey HSD, $p = 0.90$) (Fig. 4.1).

Table 4.2. Tolerance scores for diatom taxa found in > 3 of the 39 streams. Tolerance scores range from 1 (low pH optimum) to 10 (high pH optimum).

Taxa		Tolerance score	Taxa		Tolerance score
Achnanthidium	*minutissimum*	7	*G.*	*clavatum*	10
Brachysira	*brebissoni*	2	*G.*	*gracile*	2
Cocconeis	*placentula*	5	*G.*	*minutum*	10
Cymbella	*kappii*	9	*G.*	*parvulum*	6
Diatoma	*mesodon*	9	*Karayevia*	*oblongella*	6
Encyonema	*minutum*	8	*Navicula*	cf. *angusta*	10
Eunotia	*bilunaris*	2	*N.*	*lanceolata*	9
E.	*exigua*	2	*N.*	*radiosafallax*	7
E.	*implicata*	4	*N.*	*rhynchocephala*	5
E.	*minor*	4	*Nitzschia*	cf. *amabilis*	1
E.	*muscicola* var. *tridentula*	4	*N.*	*dissipata*	9
Fragilaria	*capucina*	7	*N.*	*palea*	6
F.	*capucina* var. *capitellata*	10	*N.*	*paleaeformis*	1
F.	*capucina* var. *vaucheriae*	7	*Pinnularia*	cf. *acidophila*	1
Fragilariforma	*virescens*	5	*P.*	cf. *amabilis*	3
Frustulia	*crassinerva*	3	*Planothidium*	*haynaldii*	9
F.	*rhomboides*	4	*P.*	*lanceolatum*	8
F.	*rhomboides* var. *capitata*	1	*Rossithidium*	*lineare*	8
F.	*saxonica*	3	*Surirella*	*angusta*	3
F.	*vulgaris*	6	*Tabellaria*	*flocculosa*	5
Gomphonema	*angustatum*	8	*Ulnaria*	*acus*	8
G.	*angustum*	10			

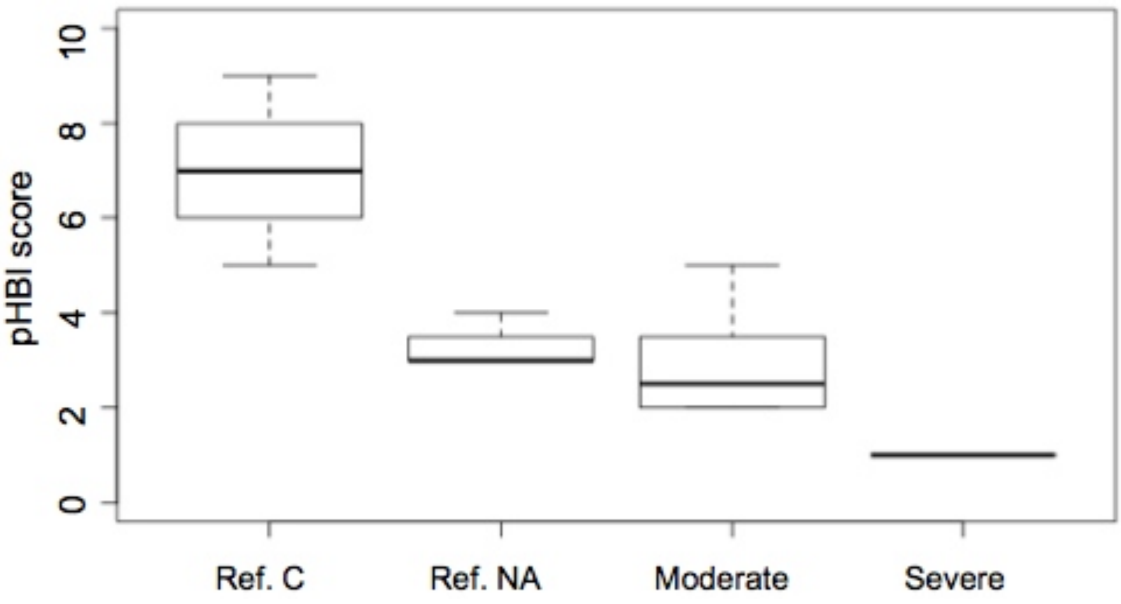


Figure 4.1. Biotic Index (pHBI) scores by AMD impact category. The bottom and top of the box are the 25th and 75th percentiles, respectively. The bold line is the median index score and the whiskers are the maximum and minimum values. Ref. C = circum-neutral reference and Ref. NA = naturally acidic reference.

4.3.3. Multimetric Index

Indicator species analysis identified eight species that were significantly ($p < 0.05$) more likely to occur in circum-neutral reference streams than the other three impact categories: *Karayevia oblongella* (Østrup) M. Aboal, *Diatoma mesodon* (Ehrenberg) Kützing, *Fragilaria capucina* var. *vaucheriae* (Kützing) Lange-Bertalot, *Gomphonema clavatum* Ehrenberg, *Gomphonema minutum* (C. Agardh) C. Agardh, *Gomphonema parvulum* (Kützing) Kützing, *Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot, and *Rossithidium lineare* (Smith) Round & L. Bukhtiyarova.

Each of the four metrics in the Multimetric Index (the Diatom-based Mine Pollution Score, DMPS) was significantly correlated with pH (Fig. 4.2). As predicted, average Bray-Curtis similarity and indicator species abundance increased with pH (Fig. 4.2C, D), and the abundance of dominant genera and acid tolerant taxa decreased with increasing pH (Fig. 4.2A, B). DMPS results ranged from 0 – 37 and were strongly correlated with pH (r =

0.88, $p < 0.0001$), specific conductivity (r = -0.74, $p < 0.0001$) and Al concentrations (r = -0.75, $p < 0.0001$).

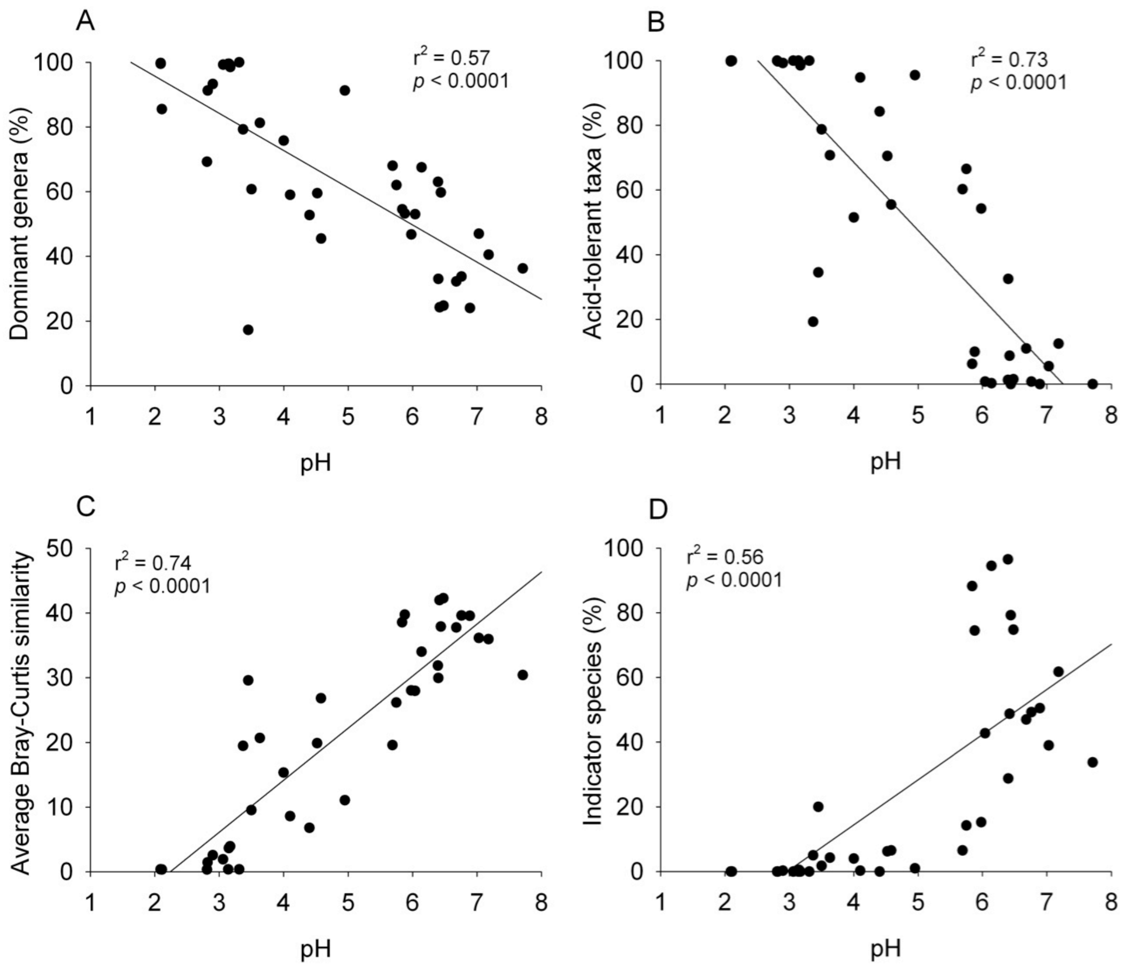


Figure 4.2. Relationship between four metrics included in the DMPS and pH at the 39 streams: relative abundance (%) of dominant genera (A), relative abundance (%) of acid-tolerant taxa (B), average Bray-Curtis similarity to circum-neutral reference streams (C), and relative abundance (%) of indicator species (D).

As with pHBI scores, DMPS results were significantly different between circum-neutral reference, moderately, and severely impacted streams (One-way ANOVA, $F_{df=3}$= 122.08, $p < 0.0001$: Tukey HSD, $p < 0.001$). There was no significant difference in index scores between naturally acidic reference and moderately impacted streams (ANOVA Tukey HSD, $p = 0.43$) (Fig. 4.3).

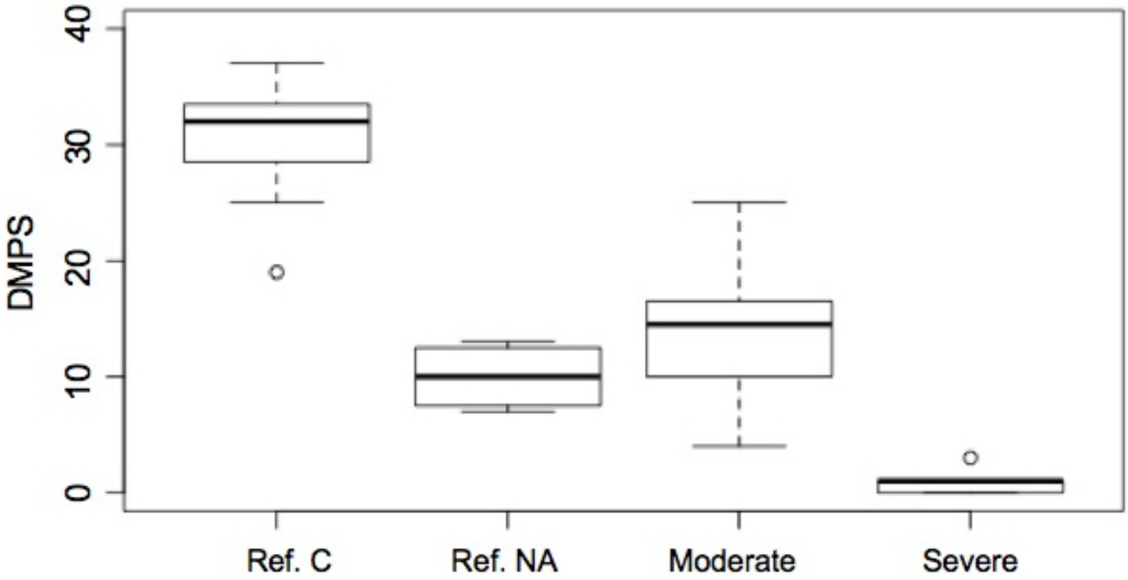


Figure 4.3. Diatom-based Mine Pollution Scores (DMPS) by AMD impact category. The bottom and top of the box are the 25^{th} and 75^{th} percentile scores, respectively. The bold line is the median index score, and the whiskers are either the maximum/minimum value or 1.5 times the interquartile range (whichever value is smaller). Open circles are outliers. Ref. C = circum-neutral reference and Ref. NA = naturally acidic reference.

4.3.4. Comparison to the AMD-DIBI

In order to test the robustness of this multimetric approach, results of the present study were compared to the AMD-DIBI developed by Zalack et al. (2010) for U.S. acid mine drainage streams. One-way ANOVA results indicated that three of the nine metrics in the AMD-DIBI were not significantly different between AMD impact categories in the present study. These were: abundance of *Cymbella, Encyonema* and *Reimeria* species ($F_{df=3}$ = 2.6605, p = 0.06), abundance of eutraphentic species, i.e. *Nitzschia* and *Navicula* ($F_{df=3}$ = 0.5455, p = 0.6545), and chlorophyll *a* ($F_{df=3}$ = 2.171, p = 0.11). A fourth AMD-DIBI metric, taxonomic richness, was not significantly different between moderately impacted streams and circum-neutral reference (ANOVA Tukey HSD, p = 0.91) and naturally acidic reference streams (ANOVA Tukey HSD, p = 0.54). Severely impacted streams did have a lower taxonomic richness (1 – 5 taxa) than each of the other three stream categories.

Two AMD-DIBI metrics use the ecological indicator values of the Kentucky Diatom Pollution Tolerance Index (KYDPTI; Kentucky Division of Water 1993): percent sensitive species and percent tolerant species. However, 39% of species observed in the present study that were identified to species level or lower were not included in the KYDPTI, including the most abundant species in circum-neutral reference streams *K. oblongella* (average relative abundance 14.7%) and the most abundant species in severely impacted streams *P*. cf. *acidophila* (average relative abundance 94.1%).

4.3.5. Comparison between the Biotic Index (pHBI) and Multimetric Index (DMPS)

DMPS and pHBI scores were strongly correlated (r = 0.955, $p < 00001$). In both indices, severely impacted streams had the lowest scores and circum-neutral reference streams had the highest (Table 4.3). Scores for both indices for naturally acidic reference and moderately impacted streams fell within the same range, and both the pHBI and the DMPS misclassified a single moderately impacted stream, Wearne Creek, as a circum-neutral reference stream.

Table 4.3. Likely range of Multimetric Index (DMPS) and single Biotic Index (pHBI) scores for each of the four stream types.

AMD impact category	DMPS	pHBI
Reference, circum-neutral	19 – 37	5 – 9
Reference, naturally acidic	4 – 18	2 – 4
Moderate	4 – 18	2 – 4
Severe	0 – 3	1

4.4. Discussion

Both the single Biotic Index (pHBI) and Multimetric Index (DMPS) were significantly correlated with water chemistry parameters and were able to categorise streams into varying degrees of AMD impact. However, neither index was able to differentiate naturally acidic streams (pH 4.0 – 5.7) from those moderately impacted by AMD (pH 3.4 – 5.8). Similar diatom communities were found in both stream types and were composed primarily of acid-tolerant *Eunotia* and *Frustulia* species. Thus, pH rather than metals (and hydroxides) would seem to be the main driver of diatom communities in these streams. If the primary goal of any assessment is to restore or monitor an AMD-impacted naturally acidic stream, then diatoms may not be appropriate indicator species. However, diatom-based indices may be especially useful in monitoring the restoration or degradation of circum-neutral reference streams. Interestingly, there were effectively no differences between the pHBI and DMPS; the two were highly correlated. The pHBI was developed based on pH optima, and all metrics in the DMPS were significantly correlated with pH. As a result, the pHBI is recommended as the preferred index for AMD monitoring, as it requires fewer analyses than the DMPS with the same level of accuracy.

The development of New Zealand specific diatom-based biotic indices may be especially valuable as there is increasing evidence that Australasia is an important region for diatom endemism (e.g. Sabbe et al. 2001, Kilroy et al. 2003, Kilroy et al. 2007). This is not surprising as significant endemism is a feature of much of New Zealand's freshwater fauna (Collier 1993, McDowall 2010). For example, *Cymbella kappii* (Cholnoky) Cholnoky is commonly regarded as a Southern Hemisphere species (Kilroy 2007). This species received a tolerance score of 9 in the present study, and is an important indicator of streams that are not impacted by AMD. Indices developed in the Northern Hemisphere that rely on ecological tolerance values of common taxa may be of limited value as they will not include Southern Hemisphere species such as *C. kappii*. Diatom genera with wide global distributions may also differ in their response to impairment between countries and regions. Local geographic factors (e.g. climate and geology) as well as physicochemical parameters determine which species may be found at a site (Vanormelingen et al. 2008). For example, *Nitzschia* and *Navicula* were important diatom genera in streams receiving AMD in the United States (Zalack et al. 2010). However, while both *Nitzschia* and *Navicula* species are common in streams in this study, with the exception of *N. paleaeformis* these genera were not indicative of AMD impacted streams in New Zealand.

Several metrics in the U.S. diatom-based multimetric index (the AMD-DIBI) developed by Zalack et al. (2010) are based on the relative abundance of diatom genera such as *Cymbella* and *Navicula*. Metrics that rely on the abundance of specific taxa may be less transferrable between regions than metrics such as diversity or similarity to reference sites. The comparison with the AMD-DIBI showed that three of the nine metrics in the AMD-DIBI were not significantly different between AMD impact categories in New Zealand. Therefore, the AMD-DIBI could not be effectively applied to New Zealand mine affected streams within this study.

The two indices developed in this study differ from a number of those developed elsewhere (e.g. Wang et al. 2005, Chessman et al. 2007, Zalack et al. 2010) in that they are based on epiphytic diatoms. In general, epilithic diatom biomass in the 39 sample streams was very low. This may be due to the high number of floods on the West Coast, which regularly scour the substrate. In 2011 Westport (a town near many of the study sites) received 2,078 mm of rainfall (NIWA 2012) compared to an annual average of 900 to 1,020 mm in the Western Allegany Plateau (McNab and Avers 1994), where a number of published U.S. studies on algae in AMD streams have occurred. In my study streams, filamentous algae and mosses were often found on large boulders that would be protected from scouring events, whereas epilithic diatoms may be washed off smaller cobbles. Epilithic diatoms are typically used in water quality monitoring (Kelly et al. 1998); however, several studies have suggested that diatoms from other habitat types may also be used. In a study comparing the use of epilithic and epiphytic diatoms, Winter and Duthie (2000) found that both communities were strongly correlated with physicochemical parameters. In a similar study, Bere and Tundisi (2011) sampled diatoms on stones, macrophytes, sand, and silt and found similar communities on each. They suggested that results of diatom-based assessment might be interchangeable among substrate types. In the present study similar genera were found as epiphytes in circum-neutral reference (e.g. *Karayevia* and *Gomphonema*) and moderately impacted streams (*Eunotia* and *Frustulia*) as were found as epiliths in previous AMD studies (de la Peña and Barreiro 2009, Zalack et al. 2010).

Both the pHBI and the DMPS incorrectly classified a single site, Wearne Creek, as a circum-neutral reference stream when water chemistry showed it to be moderately impacted by AMD at the time of diatom sampling. Wearne Creek receives AMD from a collapsed abandoned mine and the degree of mine discharge oscillates depending on storm events and further collapses within the mine. The most abundant species at this stream was *K. oblongella* (14.8% relative abundance) followed by *Frustulia saxonica* Rabenhorst (10% relative abundance). *K. oblongella* is typically more abundant in circum-neutral environments and was identified as an indicator species of circum-neutral reference streams in the present study, whereas *F. saxonica* prefers streams with pH < 5.5 (van Dam et al. 1994). *Brachysira brebissonii* R. Ross was also identified at this stream (7% relative abundance). A similar species, *Brachysira vitrea* (Grunow) R. Ross was found in greatest relative abundance at sites fluctuating between circum-neutral and acidic in the U.S., and it was suggested that this species might indicate variable pH (Verb and Vis 2000). Diatom index scores that contrast with spot water chemistry measurements coupled with the presence of *Brachysira* species may be indicative of streams that receive varying levels of AMD throughout the year.

In developing these indices samples were taken from almost all accessible AMD streams in the region. The large number of sites required to both develop and robustly test an index is a challenge often faced by researchers (Gray and Harding in press). An additional challenge is the potential variability in diatom community composition at a site both seasonally and annually (Virtanen et al. 2011). While the species that are able to tolerate severely impacted sites are constrained by extreme pH and heavy metal concentrations year-round, species in reference and moderately impacted sites may be affected by seasonal fluctuations including temperature and nutrients, as well as AMD (Bray 2007, Smucker and Vis 2011b). More work is needed to fully test these indices against an independent dataset and along a temporal gradient.

Streams receiving AMD on the West Coast of New Zealand are rare globally in that they occur in close proximity to naturally acidic brown water streams. Results indicate that diatom communities in naturally acidic streams are similar to those found in streams receiving moderate levels of AMD. This is a potential challenge for monitoring AMD-contaminated streams using diatoms in regions with natural acidity. Overall, the results of the present study suggest that diatom-based indices are a potentially valuable new tool for water managers, industry, and regulators to assist them in determining the presence and magnitude of AMD impacts in naturally circum-neutral New Zealand streams.

Chapter 5

Response of diatom communities to rapid changes in water chemistry

5.1. Introduction

AMD is a significant environmental issue faced by many countries with current and historic coal mining. Frequently, coal mining run-off can create streams with low pH, high conductivity, and elevated metal concentrations (Kelly 1988). If pH increases (either downstream or by dilution), metals may precipitate from solution, covering the streambed in metal hydroxides. Both chemical and physical stressors associated with AMD can have significant effects on in-stream biota (Hogsden and Harding 2012a). Several previous studies have investigated the diatom communities in AMD streams and their relationship to AMD water chemistry parameters (Verb and Vis 2000, de la Peña and Berreiro 2009, Zalack et al. 2010). However, in these mining systems water chemistry may change rapidly, as mining practices and intensity varies or remediation occurs. Few studies have investigated the response of diatom communities to short-term changes in the degree or intensity of mine discharge (but see Niyogi et al. 1999, DeNicola and Stapleton 2002).

The use of diatoms as indicators of water quality has become widespread (Pan et al. 1996, Atazadeh et al. 2007, Chessman et al. 2007). In particular, diatoms are increasingly recognised as useful for monitoring the effects of AMD (Luís et al. 2009, Smucker and Vis 2009, Zalack et al. 2010). One commonly cited advantage of using diatoms as biological indicators is their short generation time (Stevenson et al. 2010, Rimet 2012). Asexual cell division in diatoms is clonal, with an estimated generation time of about 12 hours (Lowe 2011). This short generation time can be compared to one generation per year for the stonefly genus *Zelandobius* Tillyard (Winterbourn 1978, Graesser 1988). Thus, diatoms may be more useful indicators of recent changes in water quality than other biomonitoring indicators. In addition to short generation times, many diatom species have narrow environmental tolerances to mine-related parameters, such as metal concentrations (von Falkenhayn 2007) and conductivity (Potapova and Charles 2003). For example, the optimal copper concentration for *Sellaphora pupula* (Kützing) Mereschkovsky is 0.31 mg/L (±1.91 mg/L) (von Falkenhayn 2007). Copper levels above this range may result in a decrease in the abundance of *S. pupula* and an increase in the abundance of more tolerant species. Autecology coupled with a rapid turnover rate allows diatoms to respond quickly to environmental changes.

Translocation experiments are a commonly used method to assess the response of diatoms to improvements or declines in water quality (Rimet et al. 2009, Morin et al. 2010, Duong et al. 2011, Lacoursière et al. 2011). In these experiments, mature biofilms on natural (e.g. cobbles) or artificial (e.g. glass slides) substrate are transferred between systems of contrasting water quality. Diatoms on the transferred substrate are sampled over time to investigate changes in community structure. Previous translocation experiments have shown contrasting results on the time required for diatom communities on transferred substrate to resemble ambient diatom communities. The response time of diatoms to a decrease in pollution levels can range from days (Wendker 1992), to weeks (Hirst et al. 2004), to months (Rimet et al. 2005). Response time may be a function of several different factors, including community composition of the recipient stream or chemical conditions at a site (Lavoie et al. 2008). Lacoursière et al. (2011) transferred diatoms grown on artificial substrate between reference and nutrient enriched streams, and calculated the difference in Eastern Canadian Diatom Index scores between transferred and *in situ* communities over time. They found that diatom communities in oligotrophic waters were less diverse and responded within a week to nutrient enrichment, whereas the taxonomically rich communities of eutrophic streams took up to four weeks to respond to nutrient reductions. While diatoms may be useful indicators of the presence or magnitude of AMD, the ability of diatom communities to respond to rapid increases or decreases in mine discharge is not well studied. Few studies have investigated diatoms communities in AMD-remediated streams, and those that have, focus on sites several years after remediation was carried out (Diamond et al. 1993, Verb and Vis 2000).

The aims of this study were to investigate diatom community response to rapid changes in AMD water quality and estimate the time required for transferred communities to resemble the ambient diatom assemblage. This was accomplished by translocating epilithic diatom communities on natural substrate between non-impacted circum-neutral, moderate AMD and severe AMD streams.

5.2. Methods

5.2.1. Study sites

Nine streams from three stream types were selected for translocations. All were located near Westport on the West Coast, South Island, New Zealand. Streams were categorised *a priori* into three stream types based on their water chemistry and the presence of iron hydroxide deposition. The three types were; 1) un-impacted reference, 2) moderately impacted by AMD with recent iron hydroxide deposition, and 3) severely impacted by AMD but with no recent iron hydroxide deposition. Three streams from each stream type were sampled over a period of 13 days in August – September 2011. All streams were at base-flow for two weeks prior to the experiment to allow for sufficient algal growth on rocks.

5.2.2. Experimental design

At each stream, 18 cobbles (approximately 8 x 12 cm) were selected. All cobbles had visible diatom growths or were slippery to the touch. The lower surface of each cobble was marked with flagging tape attached using Loctite® instant adhesive. Marked cobbles were immersed in water from the collection site and reciprocally transferred between streams. This resulted in each stream having nine cobbles from each of two other streams and a total of 162 cobbles being translocated (Fig. 5.1). Transferred cobbles were placed on the streambed in habitat protected from high flow (e.g. behind boulders or woody debris). In order to determine the starting diatom community (Day 0) of each stream, the uppermost surface of three cobbles was scraped with a stiff-bristled toothbrush and the algal slurry was combined with stream water into a 50 mL composite sample. On Days 4, 9, and 13 post-transfer, the uppermost surface of three marked cobbles from each of the other two streams was similarly sampled. In the field, all samples were preserved in 3% phosphate-buffered formalin (1 M), placed on ice and returned to the laboratory for analysis.

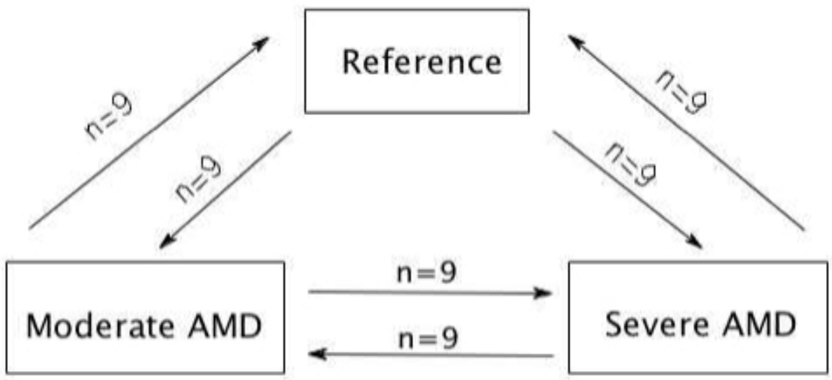


Figure 5.1. Design of cobble transfer experiment between nine streams (three reference, three moderately impacted and three severely impacted). Arrows represent the direction of transfer, and numbers represent the number of cobbles transferred.

Measurements of pH, specific conductivity and temperature were taken at each stream on four occasions; days 0, 4, 9 and 13 post-transfer using hand held meters (Eutech PC 300, Singapore). Concentrations of dissolved Al, Fe, Zn, and Mn at each stream were based on the results of previous analyses by inductively coupled plasma mass spectrometry (ICP-MS) (Chapter 3).

5.2.3. Diatom sample preparation and enumeration

In the laboratory, the 50 µL sub-sample of algal slurry was pipetted onto a slide, and a wet mount prepared. Diatoms were observed under a microscope at 1000x magnification with oil immersion. From each sample up to 400 diatom frustules were identified to the lowest possible level, primarily using the taxonomic keys of Cox (1996) and Krammer and Lange-Bertalot (1991a, b, 1997, 2008). Diatoms were categorised as dead or living at the time of collection based on the state of the chloroplast. Living frustules had healthy, intact chloroplasts and dead frustules were either empty of cellular contents or plasmolized (Fig.

5.2). A maximum of two slides from each sample were scanned for diatoms. If 400 frustules were not found on two slides, then as many as possible were counted. Identification based on the preserved field samples was sometimes uncertain. In these cases, the size and shape of a species in the preserved sample was compared with species of similar morphology in an acid-cleaned sample of the Day 0 control as described by Biggs and Kilroy (2000).

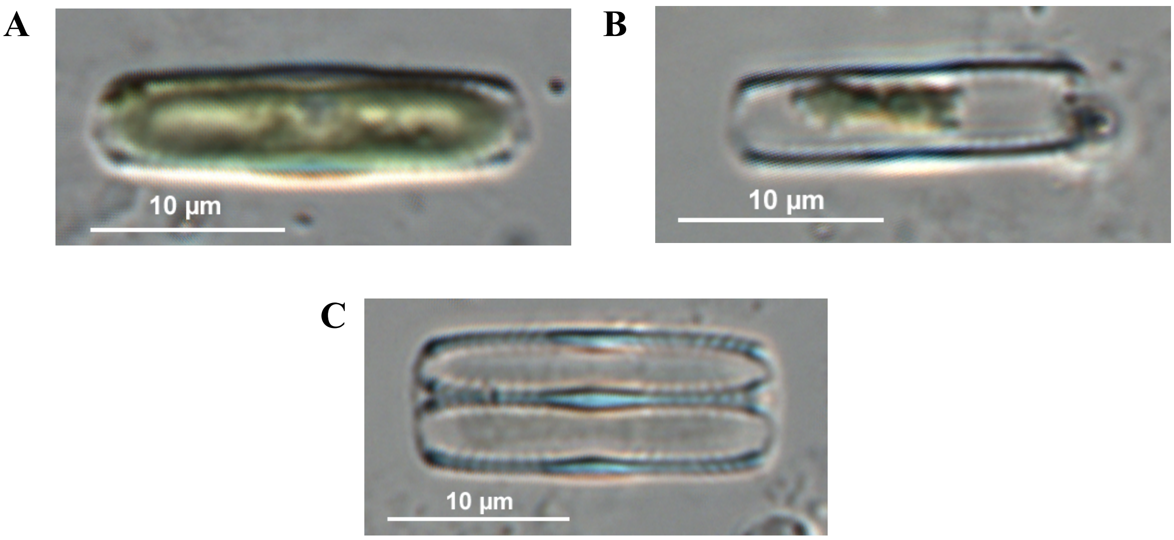


Figure 5.2. Examples of a healthy intact chloroplast that categorised the cell as living (A), a plasmolized chloroplast that categorised the cell as dead (B), and empty frustules that categorised cells as dead (C).

5.2.4. Statistical analyses

Data analysis was performed using the statistical package R (Version 2.13.0, R Development Core Team, Vienna, Austria). Water chemistry variables were compared between stream types with a multivariate analysis of variance (MANOVA). For each variable, one-way analysis of variance (ANOVA) with Tukey HSD tests were used to identify where significant differences occur. Prior to analysis, variables were assessed for normality (Shapiro-Wilk test, $p > 0.05$) and log-transformed when necessary to meet normality assumptions. To identify changes in diatom community composition over time, Detrended Correspondence Analysis (DCA) was performed on the proportion of living cells in the Day 0, 4, 9 and 13 samples using CANOCO (Version 4.5, Microcomputer Power, Ithaca, New York). Species data were square root transformed prior to analysis.

Species were categorised as acid-tolerant or circum-neutral using the ecological indicator values of van Dam et al. (1994). *Pinnularia* cf. *acidophila*, a recently described species (Krammer 2000), was not included in van Dam et al. (1994). This species was categorised as acid-tolerant for analysis (Krammer 2000, Kim et al. 2008). For each sample, the relative abundance of dead acid-tolerant and circum-neutral diatoms was calculated. A linear mixed-effect model was used to: 1) determine if there was a significant difference in the mortality rate of diatoms transferred between reference and severely impacted streams, and 2) determine if there was a significant increase in taxonomic richness over time in substrate transferred from moderately impacted to reference and severely impacted streams (Pinheiro and Bates 2000). In the model, streams were classified as a random variable and time and impact category as fixed variables.

A biotic index score based on pH tolerance (pHBI) was calculated for each sample on Days 0, 4, 9, and 13 in the reciprocal transfer between reference and severely impacted streams. Biotic index scores were calculated based on the cells categorised as living at the time of sample collection. Streams receiving a pHBI score of 1 were classified as severely impacted, 2 – 4 as moderately impacted and 5 – 9 as circum-neutral reference. Scores were rounded to the nearest whole number. The methods used to develop the pHBI as well as tolerance scores for individual taxa are provided in Chapter 4.

5.3. Results

5.3.1. Water chemistry

Water chemistry varied markedly between streams. Specific conductivity ranged from 31 – 2,285 μS_{25}/cm and pH from 2.1 – 7.3. A wide range of metal concentrations was also observed (Al: 0.1 – 59.16 mg/L, Fe: 0.04 – 11.39 mg/L, Zn: 0.02 – 2.20 mg/L, Mn: 0.00 – 1.99 mg/L). Specific conductivity, temperature, and pH varied within a stream across sampling dates. For example, specific conductivity at one reference stream ranged from 40 to 65 μS_{25}/cm between sampling dates.

As expected, specific conductivities and metal concentrations were significantly different between the three stream types (MANOVA, $F_{df=7} = 14.73$, $p = 0.025$) with severely AMD-impacted streams having very high conductivities, Al, Fe, Zn, and Mn concentrations (Table 5.1). Temperature was also significantly different between stream types, with higher average temperatures observed in severely impacted streams. Although MANOVA statistics could not be calculated on average pH, the range of pH values at each stream reflected the three stream types; the lowest pH was observed in severely impacted streams and the highest pH in reference streams (Table 5.1).

Table 5.1. Means (±SEM) and univariate ANOVA statistics of specific conductivity, temperature, and metal concentrations in reference, moderately impacted and severely impacted streams. The range of values in each category is provided for pH. Significant differences ($p < 0.05$) using Tukey's HSD are indicated by different superscript letters.

	Reference	Moderate AMD	Severe AMD	F	*P*
pH	6.2 – 6.8	2.9 – 5.0	2.5 – 2.7	-	-
Conductivity (μS_{25}/cm)	55^{a} (±7.8)	507^{b} (±237)	1619^{b} (±222)	52.65	< 0.001
Temperature (ºC)	8.2^{a} (±0.46)	8.5^{a} (±0.48)	15.1^{b} (±1.15)	18.39	< 0.01
Al (mg/L)	0.12^{a} (±0.04)	$18.65^{a,b}$ (±9.71)	46.30^{b} (±6.49)	26.04	< 0.01
Fe (mg/L)	0.07^{a} (±0.03)	$4.69^{a,b}$ (±2.47)	10.46^{b} (±0.82)	17.14	< 0.01
Zn (mg/L)	0.03^{a} (±0.004)	0.46^{a} (±0.22)	1.54^{b} (±0.36)	18.83	< 0.01
Mn (mg/L)	0.00^{a} (±0)	0.33^{a} (±0.19)	1.48^{b} (±0.34)	18.89	< 0.01

5.3.2. Taxonomic richness and composition of the resident diatom community

Only two species were recorded on substrate from severely impacted streams: *Nitzschia paleaeformis* Hustedt (relative abundance 0 – 26%) and *Pinnularia* cf. *acidophila* Hofmann & K. Krammer (relative abundance 74 – 100%). One moderately impacted stream had a similar community to severely impacted streams with *P.* cf. *acidophila* at 95% relative abundance and *N. paleaeformis* at 5% relative abundance. For the duration of the experiment, this stream had lower pH (2.7 – 3.0) and higher specific conductivity (average 950 μS_{25}/cm) than each of the other two moderately impacted streams. In the remaining two moderately impacted streams four species were found in extremely low densities. A single dead valve of *Eunotia* sp. was identified at the second moderately impacted stream and one dead valve each of *Cocconeis placentula* Ehrenberg, *Gomphonema parvulum* (Kützing) Kützing and *Encyonema* sp. was identified at the third moderately impacted stream.

Taxonomic richness was greatest in reference streams, ranging from 10 – 17 taxa per stream. *Rossithidium lineare* (W. Smith) Round & Bukhtiyarova was the most abundant species in reference streams (average relative abundance 36%) followed by *Diatoma mesodon* (Ehrenberg) Kützing (average relative abundance 17%) and *G. parvulum* (average relative abundance 16%). *N. paleaeformis* and *P.* cf. *acidophila* were absent from reference streams.

5.3.3. Mortality

Diatom mortality increased during the 13 days in response to both the addition to and removal from AMD. In the transfer from severe AMD to reference streams, an average of 61% of acid-tolerant cells died by Day 13 (Fig. 5.3A). In the reverse transfer, an average of 96% of circum-neutral cells died by Day 13 (Fig. 5.3B). However, both acid-tolerant and circum-neutral cells died at similar rates (as indicated by the slope of the regression line, Figs 5.3A, B) in the reciprocal transfer between reference and severely impacted streams (mixed-effect model, $F_{df=16} = 2.699$, $p = 0.120$). Diatom mortality also increased in the transfer from reference to moderately impacted streams: an average of 71% circum-neutral cells died by Day 13 (Fig. 5.3B). However, there was no significant increase in mortality in the transfer from severely to moderately impacted streams (Fig. 5.3A).

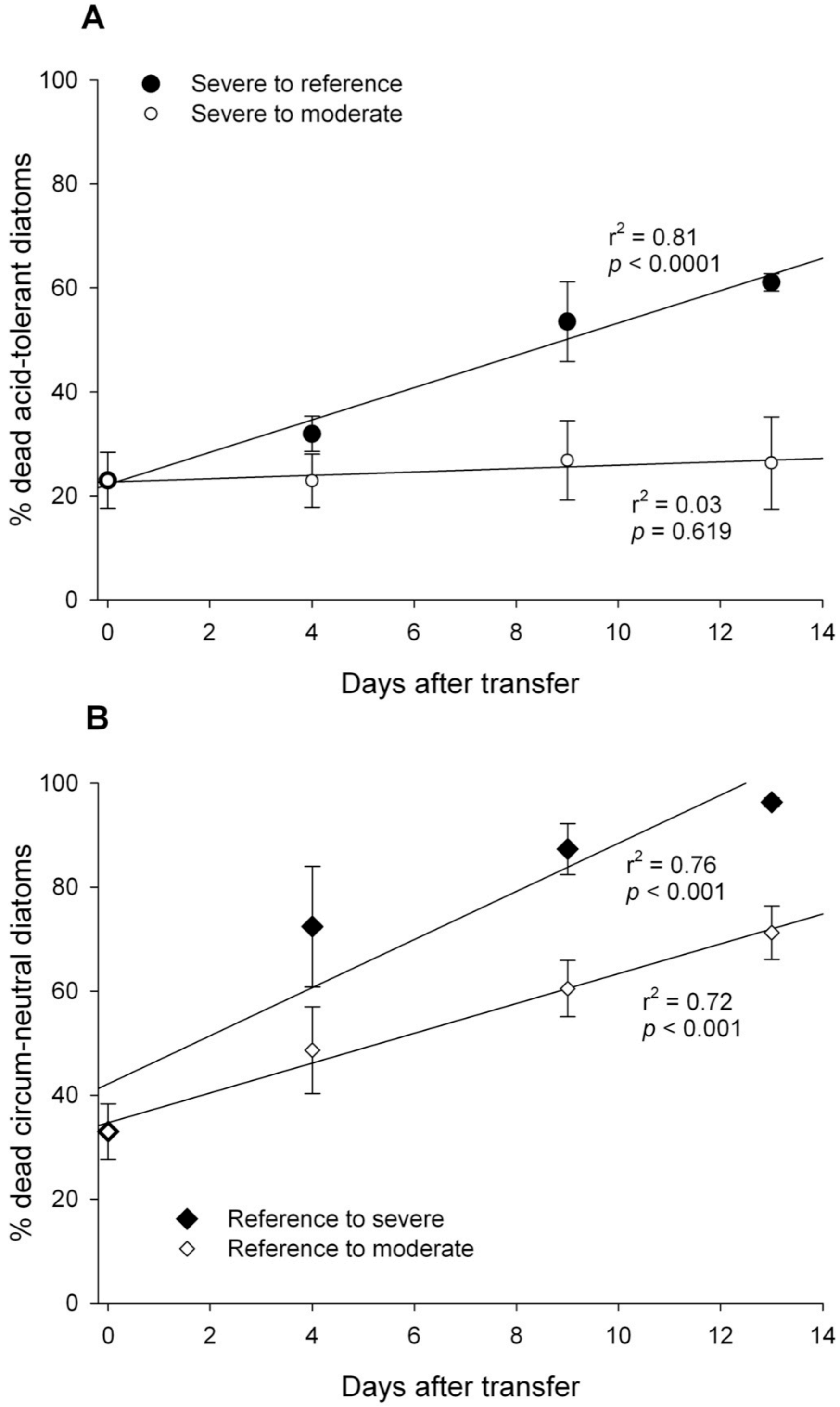


Figure 5.3. Mean relative abundance (± SE) of dead acid-tolerant (A) and circum-neutral (B) diatom cells at 0, 4, 9 and 13 days post-transfer. Statistics shown are regression significance. $N = 3$ streams.

5.3.4. Colonisation

Diatoms rapidly colonised cobbles transferred from moderately impacted to reference streams (Fig. 5.4A). All cobbles from moderately impacted streams had a thin coating of iron hydroxide that was retained throughout the experiment, but this did not prevent diatoms from colonising the cobbles after they were transferred to reference streams. There was a significant increase in taxonomic richness between Day 0 and 4 (mixed-effect model, $F_{df=2} = 41.29$, $p = 0.02$) (Fig. 5.4A); however, after Day 4 no significant change in richness occurred ($F_{df=5} = 0.02$, $p = 0.89$). *Diatoma mesodon* (Ehrenberg) Kützing was the most abundant species (52 – 66% on Day 13) to colonise substrate from the two moderately impacted streams with low diatom density. In the third moderately impacted stream, *P*. cf. *acidophila* continued to dominate the living sample after 13 days. There was no significant increase in taxonomic richness on substrate transferred from moderately impacted to severely impacted streams over the experiment ($F_{df=8} = 2.97$, $p = 0.12$) (Fig. 5.4B).

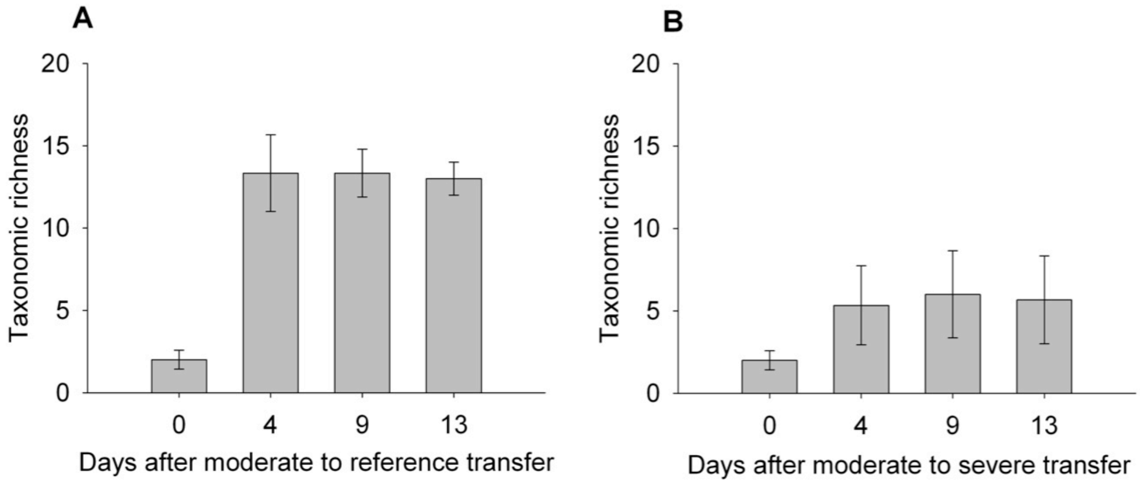
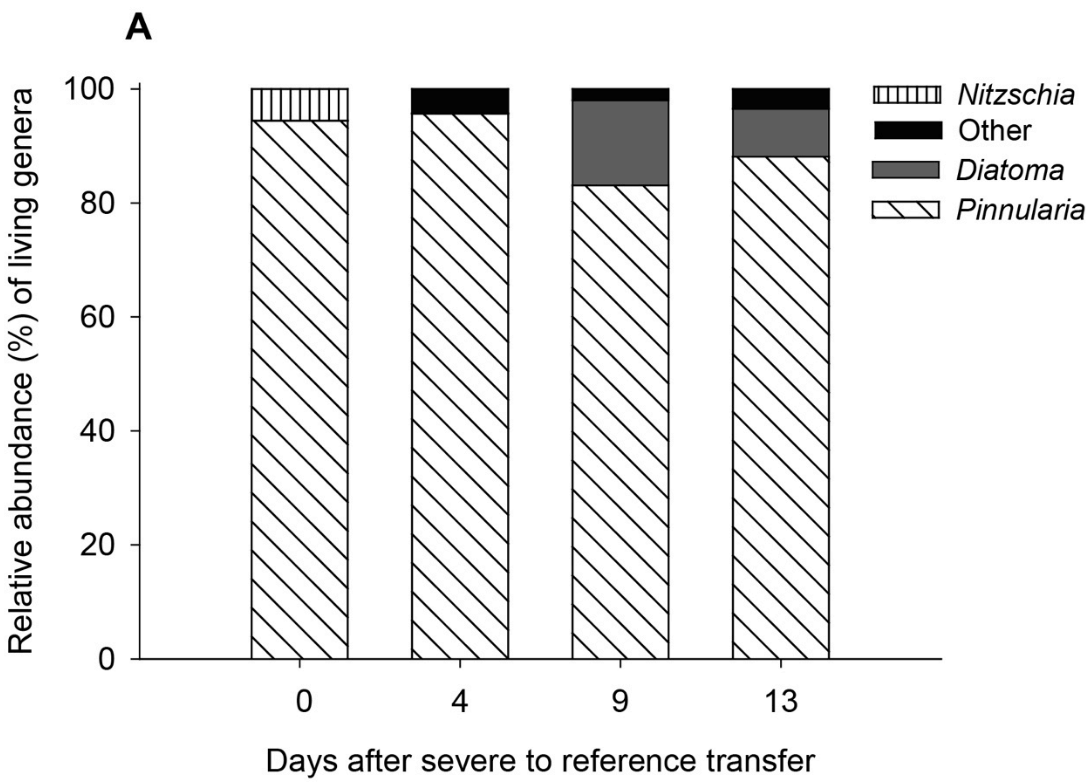


Figure 5.4. Mean taxonomic richness (± SE) (including both living and dead diatoms) in the transfer from moderately impacted streams to both reference (A) and severely impacted (B) streams. *N* = 3 streams.

Relatively few diatoms colonised cobbles following the severe AMD to reference transfer. The average relative abundance of living circum-neutral species ranged from 0% before transfer, to 12% on Day 13. Of the living diatoms present, *P.* cf. *acidophila* continued to dominate on Days 4, 9 and 13 (Fig. 5.5A). In general, the transferred diatom community remained more similar to those found in severely impacted streams than to reference streams throughout the duration of the experiment (Figs 5.5A and 5.6).

In contrast, the reverse transfer (into severe AMD streams) resulted in significant changes in diatom community composition. *P*. cf. *acidophila* quickly colonised cobbles and was found at an average relative abundance of 83% by Day 13 (Fig. 5.5B). The DCA plotted reference streams near the left of Axis 1, and severe AMD streams on the right of Axis 1 (Fig. 5.6). During the experiment, the diatom community transferred from reference streams became increasingly similar to the resident diatom community of severely impacted streams. By Day 13, transferred diatom communities were plotted near the resident communities of severely impacted streams (Fig. 5.6).



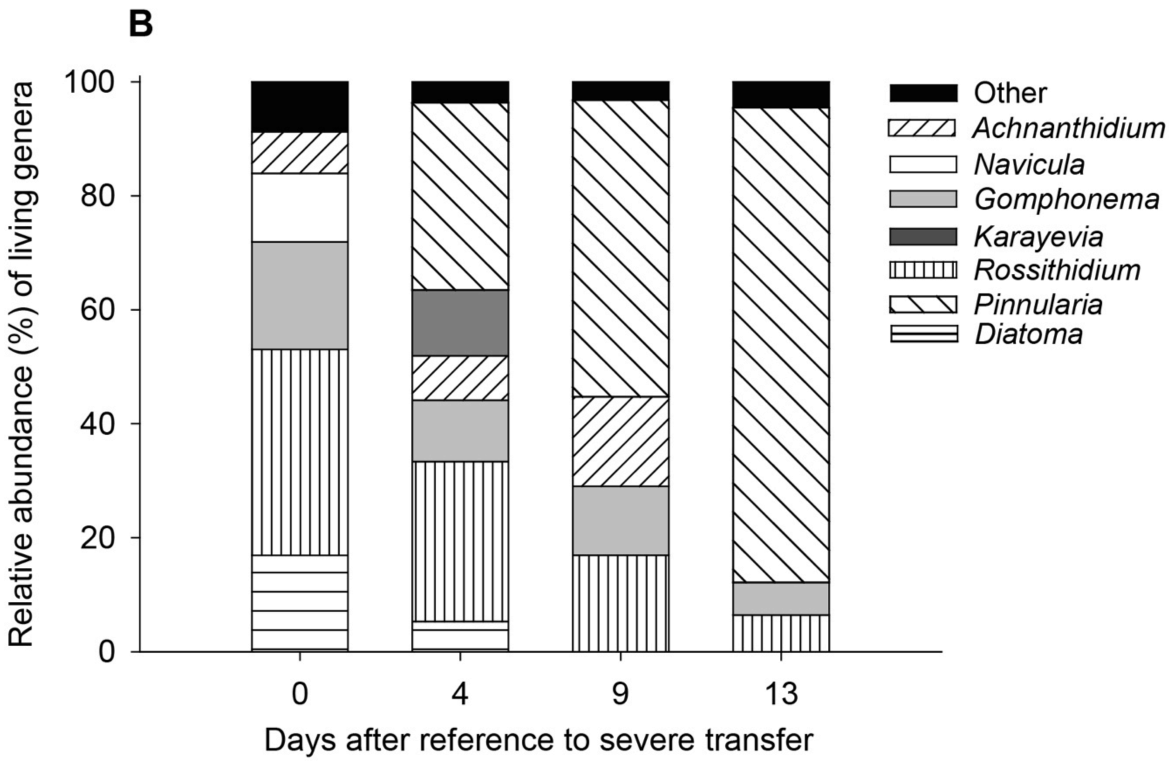


Figure 5.5. Average relative abundance of living diatom genera 0 – 13 days after the transfer from severe AMD to reference streams (A) and reference to severe AMD streams (B). Other genera include those found at ≤ 5% relative abundance. N = 3 streams.

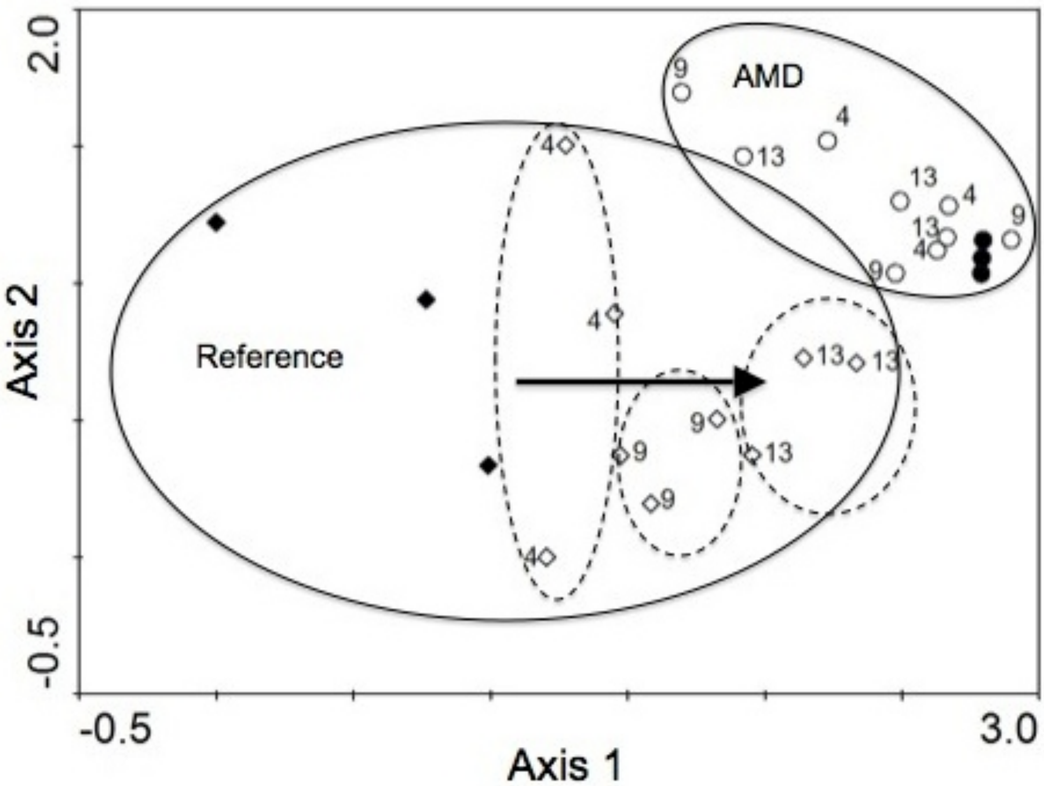


Figure 5.6. DCA biplot of the living diatom community in the transfer between reference and severe AMD streams. Black diamond = resident reference community, black circle = resident severe AMD community, open diamond = reference to severe transfer, and open circle = severe to reference transfer. The numbers 4, 9 and 13 represent the number of days post-transfer.

5.3.5. Biotic Index scores (pHBI)

Translocation of epilithic diatoms from reference to severely impacted streams resulted in a rapid decline in pHBI scores over the two weeks (Fig. 5.7A). Initially, epilithic diatom communities at each of the three reference streams scored a pHBI of 7 (indicative of un-impacted, circum-neutral streams). By Day 13, pHBI scores had declined to between 1 and 2, placing them in the category of moderately to severely impacted by AMD (Fig. 5.7A). In contrast, resident diatom communities of severely impacted streams received a pHBI score of 1. By Day 13, diatom communities of cobbles transferred from severely impacted to reference streams were categorised as moderately to severely impacted by AMD (Fig. 5.7B).

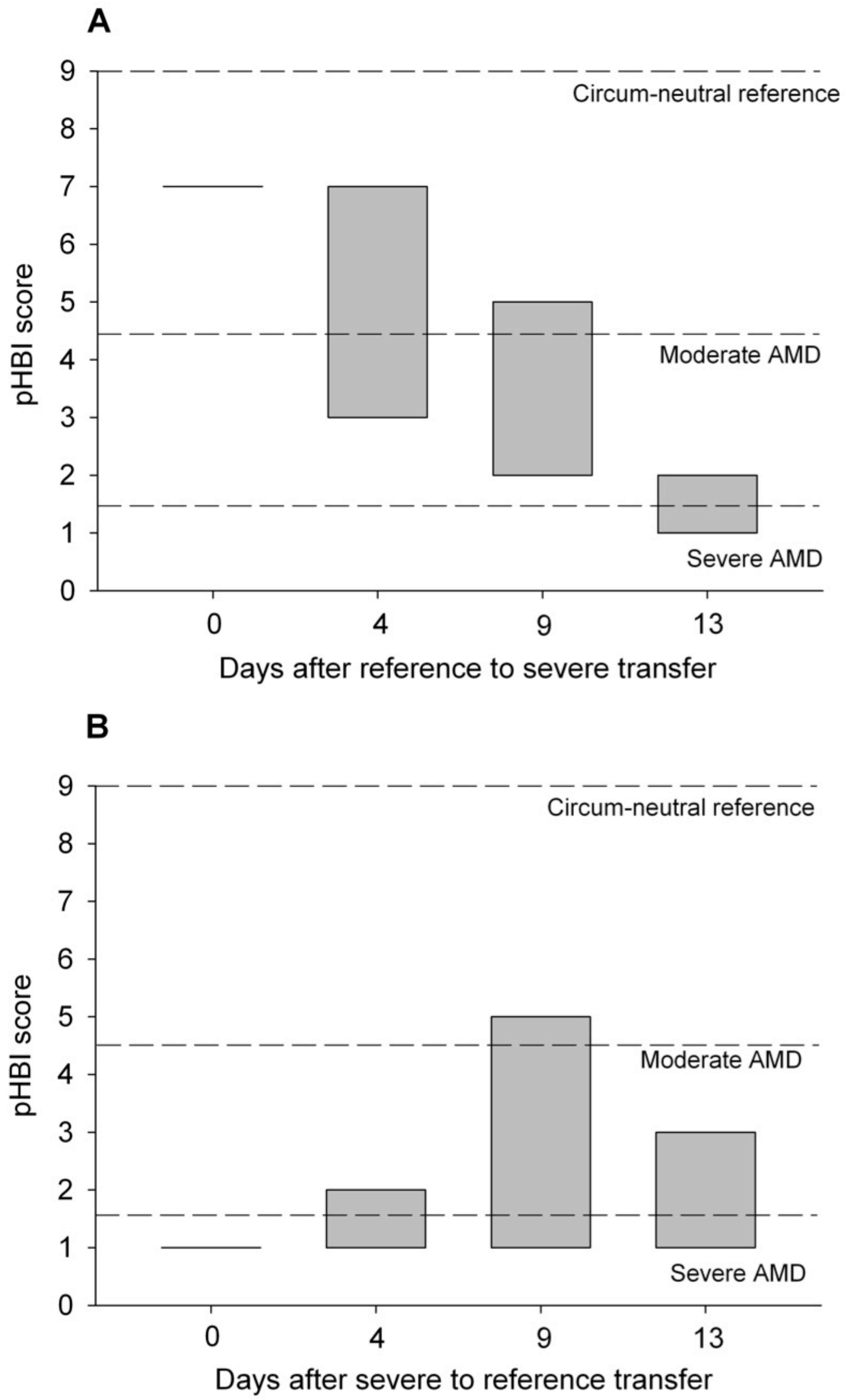


Figure 5.7. Biotic Index scores (pHBI) in the transfer from reference to severe streams (A) and severe to reference streams (B). Bars are maximum and minimum values. Dashed lines separate AMD impact categories as defined by pHBI scores.

5.4. Discussion

Translocation experiments can be a powerful tool in providing greater understanding of a community's resistance and resilience to pollution (Tolcach and Gómez 2002). Results of the present study show that epilithic diatom communities responded surprisingly quickly to marked changes in the level of mine discharge. Diatom mortality increased rapidly in response to sudden exposure to AMD waters. In streams impacted by moderate levels of AMD, pH is the overriding factor in structuring diatom community composition (see Chapter 3). In severely impacted streams, mortality is most likely due to some combination of the effects of pH and metal toxicity. While significant mortality also occurred in the transfer from severe to reference streams, colonisation rates were low and as a result there was no obvious change in diatom community composition. Overall, results indicate that diatom species are sensitive to the addition of AMD but slower to respond to the removal of AMD.

This experiment tested diatom community response to changes in water quality over a period of two weeks. In contrast, many algae translocation experiments in the literature run for 4 – 8 weeks (e.g. Rimet et al. 2009, Morin et al. 2010, Duong et al. 2011). In general, long-term translocation studies begin sampling the diatom community two to four weeks after the initial transfer and as a result are not able to observe a rapid change in community composition. Frequent sampling over a short time-scale allowed me to observe significant mortality following the transfer to both AMD and reference streams as quickly as four days post-transfer. Circum-neutral species experienced near 100% mortality after 13 days in severely impacted streams. In the reverse transfer, an average of 61% of cells of acid-tolerant species had died by Day 13. Diatom communities have been shown elsewhere to be highly responsive to changes in both pH (Planas et al. 1989, Hirst et al. 2004), and metals (Ivorra et al. 1999, Gold et al. 2002). For example, Ivorra et al. (1999) recorded a dramatic shift in community composition after two weeks in a stream with high Zn and Cd concentrations, from a community characteristic of low-metal environments to one dominated by metal tolerant taxa, such as *Neidium ampliatum* (Ehrenberg) Krammer and *Pinnularia* sp. In the present study, significant changes in mine discharge (i.e. from reference to severely impacted and vice versa), resulted in an increase in the relative abundance of dead circum-neutral or acid-tolerant species. A less drastic decrease in AMD levels, from severe to moderate, did not result in significant changes in diatom mortality. Acidobiontic species such as *P.* cf. *acidophila* and *N. paleaeformis* are characterised by an optimal pH < 5.5 (van Dam et al. 1994). As all moderately impacted streams fell within this optimal range, pH may need to exceed 5.5 before significant mortality would be observed.

Mortality was quantified by categorising diatoms as living or dead at the time of sample collection, which is a technique not previously used in algae translocation experiments. For example, in one of the few experiments to use natural substrate, Hirst et al. (2004) transferred cobbles with mature algal biofilms between streams of contrasting acidity and identified changes in diatom community composition over a period of 12 days. The authors acid-cleaned their samples and as a result were not able to differentiate living from dead cells. It is common practice to remove cellular contents by acid-cleaning diatom samples prior to identification (Kelly et al. 1998, Gillett et al. 2011). In bioassessment studies, results may be similar when calculated from living diatoms or the entire community (i.e. both living and dead) (Gillett et al. 2009). However, when the aim is to investigate changes in diatom mortality over a short-time span, individuals should ideally be categorised as living or dead at the time of sample collection (Medley and Clements 1998). Diatoms remain attached to the substrate after dying and thus a sample will contain both living and dead individuals. In the present study, an abundance of dead *P.* cf. *acidophila* cells remained attached to the substrate transferred from AMD to reference streams throughout the duration of the experiment. If mortality were based simply on the relative abundance of this species in an acid-cleaned sample, I would not have observed an increase in *P.* cf. *acidophila* mortality over time.

As with mortality, colonisation of new taxa also plays an important role in structuring the diatom community following changes in the chemical environment. The transfer of substrate from reference to severely impacted streams resulted in acid-tolerant species such as *P.* cf. *acidophila* rapidly colonising the substrate and dominating the living sample after 13 days. As a result, the diatom community on the transferred substrate became increasingly similar to the ambient diatom community of severely impacted streams. This change in community structure occurred relatively quickly; *P.* cf. *acidophila* made up a majority of the living sample within nine days. In contrast, when substrate was transferred from severely impacted to reference streams the translocated communities did not resemble the ambient diatom community after 13 days. While species such as *Diatoma mesodon, Rossithidium lineare* and *Achnanthidium minutissimum* (Kützing) Czarnecki began to colonise the transplanted substrate, the relative abundance of living taxa typical of circum-neutral environments was minor compared to that of *P.* cf. *acidophila.* There was no obvious improvement in pHBI scores 13 days following transfer; scores remained characteristic of streams moderately to severely impacted by AMD. In a similar experiment, Hirst et al. (2004) transferred rocks between two streams of contrasting acidity. *A. minutussium* dominated the circum-neutral stream and *Eunotia exigua* (Brébisson ex Kützing) Rabenhorst the acidic stream. *E. exigua* approached ambient abundance 12 days after transfer into the acidic stream. In the reverse transfer, the abundance of *A. minutissimum* was well below ambient values after 12 days. These results, as well as the results of the present study, indicate that diatom communities may respond to a decline in water quality more rapidly than an improvement.

Several previous studies have found that diatom communities respond faster to an increase in pollution levels than to pollution amelioration (Tolcach and Gómez 2002, Lacoursière et al. 2011). For example, in one of the earliest algae translocation experiments, Iserentant and Blancke (1986) transferred substrate between polluted and non-polluted streams and identified changes to diatom community composition over time. They found a significant shift in community structure within two weeks after transfer from the reference to polluted stream. However, after 45 days the transfer to the non-polluted stream showed little effect. Rimet et al. (2005) suggested it may take up to 60 days to observe significant changes in the diatom community following an improvement in water quality. Under natural conditions, the response time is likely to be even longer than that observed under simulated conditions (Rimet et al. 2009). In the present study, biofilms were transferred directly to a site where species tolerant of the environment were found. In a real world scenario, diatoms must colonise the new environment either through direct means such as drifting from un-polluted reaches or indirect means such as wind, birds, fish, or human activities (Kristiansen 1996). This potential lag in response time has significant implications for using diatoms as bioindicators of AMD remediation. While mortality occurs rapidly, colonisation of taxa characteristic of an improvement in water quality may be slow to occur. It is crucial to estimate changes in the relative abundance of dead acid-tolerant species as well as assessing community composition when monitoring AMD remediation attempts in which water chemistry is expected to change rapidly.

One possible explanation for the delay in colonisation observed at the reference streams is the environmental requirements of early successional species. Small, adnate species such as *A. minutissimum* quickly occupy available space and are typically the first species to colonise substrate after a scouring event (Stevenson and Bahls 1999). High cell densities of *P.* cf. *acidophila*, also an adnate species, were present on transferred substrate throughout the study period. This lack of free space may have limited the ability of pioneer species such as *A. minutissium* and *R. lineare* to establish populations on transferred substrate. In contrast, high profile (i.e. erect, filamentous, stalked, or chain-forming (Berthon et al. 2011)), mid-successional species such as *D. mesodon* require less free space for attachment, and began to colonise the substrate nine days post-transfer. When the epilithon is dominated by adnate species, *A. minutissimum* can attach to the surface of high profile species (Manoylov 2009). A significant increase in the abundance of circum-neutral species may have occurred shortly after Day 13, as *D. mesodon* become more abundant and dead *P.* cf. *acidophila* cells continued to slough from the substrate. In the reverse transfer, *P.* cf. *acidophila* rapidly colonised transferred substrate. While there is no literature on the colonisation rate of this species, the results of the present study indicate that if *P.* cf. *acidophila* were able to reach a stream recently impacted by AMD, it could rapidly establish a population before the resident community was fully removed from the substrate.

Along with chemical stress typically associated with AMD, changes in the physical environment may also impact diatom mortality and colonisation. The deposition rate of metal oxides is a major factor in controlling periphyton biomass, by smothering algae, decreasing light availability, and destabilising the substrate (McKnight and Feder 1984, Niyogi et al. 1999). Niyogi et al. (1999) experimentally diverted mine discharge in a U.S. stream, and found the deposition rate of metal precipitate was the dominant factor in determining *Ulothrix* sp. biomass. In the present study, recent iron hydroxide was present in all three moderately impacted streams, two of which had extremely low diatom cell densities. Diatoms were able to colonise the substrate once they were re-located to water chemistry where new iron hydroxide was not produced. *D. mesodon*, an araphid species that lacks significant motility, was the most abundant species to colonise cobbles coated with iron hydroxide in reference streams. As a high profile species, *D. mesodon* was able to attach to the substrate through a thin layer of adherent iron hydroxide that was present throughout the study period. In an environment where iron hydroxide is continually produced, *D. mesodon* may be buried under the precipitate or unable to attach to the substrate. If the negative chemical effects of AMD are treated and new iron hydroxide is not produced, epilithic diatoms may be able to colonise before old deposits of iron hydroxide are completely removed (DeNicola and Stapleton 2002).

This translocation experiment confirmed that AMD plays a major role in structuring the epilithic diatom community, which is in agreement with several previous studies (Luís et al. 2009, Smucker and Vis 2009, Zalack et al. 2010). Severely impacted streams were dominated by one or two acid-tolerant species, whereas reference streams had a rich diatom flora typical of circum-neutral environments. Furthermore, both acid-tolerant and circum-neutral diatoms were highly responsive to sudden changes in water chemistry. Despite the obvious increase in mortality, diatom community composition remained significantly different from the ambient diatom community found in un-impacted reference streams. In an AMD remediation context where a stream has been treated to raise the pH and remove metals, the availability of diatom species to recolonise the remediated stream in the short-term may be limited. This potential delay in response time is important to consider when incorporating diatoms into monitoring restoration of AMD streams, as the diatom community may take weeks to months to fully reflect an improvement in environmental condition.

Chapter 6

Conclusions

6.1. Diatom communities across an AMD gradient

The primary aim of this thesis was to determine if diatoms could be used as indicators of the presence and magnitude of acid mine drainage on the West Coast, South Island, New Zealand. Although algae in general have been studied by several workers in AMD-contaminated New Zealand streams (Winterbourn et al. 2000, Novis 2006, Bray et al. 2008), few studies have investigated diatoms, despite overseas literature indicating that they may be powerful tools for monitoring this impact within aquatic ecosystems (Zalack et al. 2010).

Results of my 39-stream survey confirmed the potential utility of diatoms as indicators of AMD within New Zealand. Epiphytic diatom communities were significantly different between circum-neutral reference, moderately impacted (pH: 3.4 – 5.8; specific conductivity: 43 – 313 μS_{25}/cm) and severely impacted streams (pH: 2.1 – 3.3; specific conductivity: 931 – 1,919 μS_{25}/cm) (Fig. 6.1). Species such as *Gomphonema minutum*, *Planothidium lanceolatum*, and *Rossithidum lineare* were characteristic of circum-neutral reference streams and were typically absent or found in low relative abundance in AMD-contaminated streams. These species may be useful indicators of streams that do not receive mine discharge. Moderately impacted streams had a diatom community composed primarily of acid-tolerant *Eunotia* and *Frustulia* species. My results showed that diatom communities in these West Coast streams are strongly structured by pH, and at moderate levels of AMD (pH 3.4 – 5.8), taxonomic richness may still be high. These findings were consistent with overseas studies that have found relatively high taxonomic richness in moderately impacted streams and low richness in severely impacted streams (Smucker and Vis 2009, de la Peña and Barreiro 2009). DeNicola (2000) suggest that at a threshold near pH 3.5 a limited number of specialist acid-tolerant species are able to survive. My results confirm those of DeNicola (2000). Taxonomic richness decreased markedly at a threshold of pH 3.4, below which all streams were dominated by a single species, *Pinnularia* cf. *acidophila*. This is particularly interesting because despite other work on algae in AMD in New Zealand, none to my knowledge have noted the dominance of *P.* cf. *acidophila*. An abundance of *P.* cf. *acidophila* seems to indicate that a stream has crossed a threshold from moderately to severely impacted by AMD. As predicted, when substrate was transferred from severely impacted to circum-neutral reference streams, the abundance of dead *P.* cf. *acidophila* cells quickly increased. In monitoring remediation attempts of severely impacted streams using diatoms, an increase in the relative abundance of dead *P.* cf. *acidophila* cells would be a powerful indicator of an improvement in water quality. Its distinct striae pattern, shape, and size distinguish it from other *Pinnularia* species and make it easy to identify. Morphologically similar species such as *Pinnularia acidophila* and *Pinnularia acoricola* were not observed in my study streams. More work is needed to compare these three *Pinnularia* taxa and determine if *P.* cf. *acidophila* is a new species.

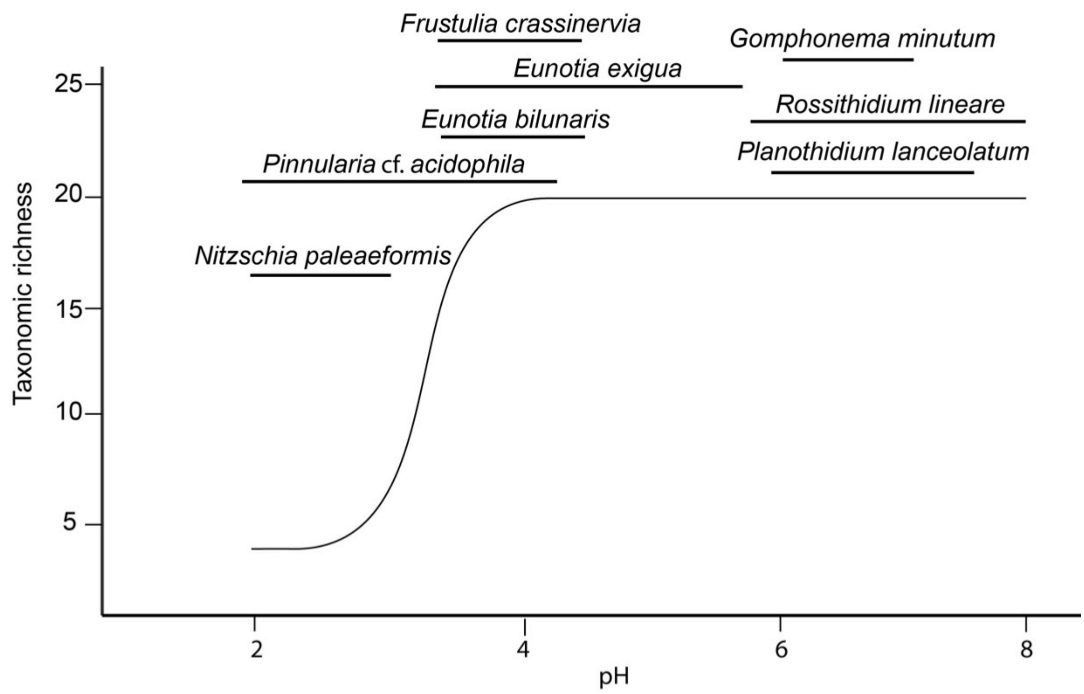


Figure 6.1. The relationship between diatom taxonomic richness and pH. Common taxa are listed along with the pH range in which they were found in > 5% relative abundance.

6.2. Diatom communities in naturally acidic streams

There is limited literature on diatoms in naturally acidic streams. As a result, it was not possible to adequately determine "reference" diatom communities for naturally low pH from international literature. The literature that is available, primarily from the U.S. (Passy et al. 2006, Zampella et al. 2007) and Sweden (Andrén and Jarlman 2008), suggests that *Eunotia* species are prevalent in naturally acidic streams. In these regions, streams may also be impacted by anthropogenic acidification as a result of acid precipitation (Johansson et al. 1995, Galloway 2001). In contrast, acid precipitation is not a major environmental concern within New Zealand (Holden and Clarkson 1986). Sampling naturally acidic streams on the West Coast of the South Island allowed me to investigate diatom communities in the absence of anthropogenic acidity.

Diatom communities in my naturally acidic study streams were composed primarily of acid-tolerant *Eunotia* and *Frustulia* species. Surprisingly, the diatom communities of naturally acidic and moderately AMD-impacted streams were similar. For example, *Eunotia* cf. *incisa* was found in 51% relative abundance in Rapid Tributary, a naturally acidic stream, and at 36% at Rapid Creek, a moderately impacted stream. Rapid Tributary and Rapid Creek both have a low pH (4.1 and 3.5, respectively) but different metal concentrations (0.16 mg/L and 18.91 mg/L of Fe, respectively). This suggests that pH is the dominant environmental factor in structuring the diatom community, and diatoms are able to tolerate moderate conductivities and metal concentrations without a corresponding shift in community composition. As a result, there was no significant difference in pHBI scores between the two stream types, indicating that the pHBI could not be used to monitor the effects of moderate AMD on diatom communities in naturally acidic streams. This represents a potential challenge for incorporating diatoms into AMD monitoring within New Zealand.

6.3. Diatom community response to fluctuating water chemistry

Diatoms may be used to indicate fluctuating water chemistry at a site that would otherwise be missed by very short-term water chemistry measurements (e.g. spot samples) and by much longer-term tools (e.g. benthic invertebrates). For example, two streams sampled within this study, Rapid and Wearne Creek, receive varying concentrations of AMD throughout the year. Despite this variable AMD, taxonomic richness was high in both streams and species typical of both circum-neutral and acidic environments were found. Circum-neutral species may colonise the stream following an increase in pH, and die but remain attached to the substrate when pH decreases. In addition, of the 39 sample streams, *Brachysira brebissonii* was present in greatest relative abundance in Rapid Creek, followed by Wearne Creek. Van Dam et al. (1994) classify this species as acidophilous, which is in agreement with the pH tolerance score of 2/10 it received in the present study. While typically associated with acidic environments, it has been suggested that *Brachysira* species are indicative of oscillating pH (Verb and Vis 2000). An abundance of *Brachysira* species as well as high taxonomic richness produced by a combination of circum-neutral and acid-tolerant species may confirm fluctuating water chemistry at a site.

Along with taxonomic richness and community composition, diatom mortality rate also responded to a rapid change in water quality. An increase in the relative abundance of dead cells was observed after just four days following the transfer from circum-neutral reference to AMD streams. In the absence of a large scouring event, dead cells may remain attached to the substrate for more than two weeks. An abundance of dead circum-neutral cells in a diatom sample may indicate a recent spike in the volume of AMD. While mortality of circum-neutral species increased in response to the addition of AMD, and acid-tolerant species to the removal of AMD, diatoms were slow to colonise substrate following an improvement in water quality. As a result, a significant increase in pHBI scores was not observed after two weeks in un-polluted reference streams. In the short-term, a change in the relative abundance of dead circum-neutral species may be a more appropriate indicator of variable water quality than a shift in community composition. Few studies distinguish living from dead cells at the time of sample collection, and the results of my work indicate that this type of analysis is especially useful in identifying diatom community response to rapid changes in water quality.

6.4. Limitations of the present study and future recommendations

While results suggest that naturally acidic streams have diatom communities similar to those moderately impacted by AMD, only four naturally acidic streams were sampled. Future research should focus on sampling diatoms in a large number of naturally acidic systems in an attempt to either confirm the results of the present study or identify acid-tolerant species that are unable to tolerate heavy metals. A translocation experiment in which algal biofilms are transferred between naturally acidic and moderately impacted streams of similar pH but contrasting heavy metal concentrations is one possibility. There are also New Zealand streams, in the Red Hills range, that have naturally high metal concentrations and a circum-neutral pH (Hogsden and Harding 2012b). Comparing diatom communities in AMD, naturally acidic, and naturally high metal streams would be useful in separating pH and metal tolerances of common species.

This study was the first to develop a diatom-based biotic index, the pHBI, for use within New Zealand. In developing the pHBI, pH tolerance scores for 43 taxa were calculated. Accurate species identification is a major challenge when diatom-based indices are used for monitoring (Prygiel et al. 2002). With this in mind, I created a detailed photographic guide to all 43 taxa included in the pHBI, as well as a selection of rare taxa. This guide should assist identification of common diatoms on the West Coast and calculation of pHBI scores. However, more work is needed to fully test the pHBI against an independent dataset and assess the robustness of pHBI scores across a temporal gradient before it can be used in monitoring AMD-contaminated streams. In the present study, all streams categorised as severely impacted were located just north of Westport in streams draining the Stockton Plateau. Streams classified as severely impacted ($pH < 3.4$) in areas such as Reefton and Greymouth should also be sampled to determine if the pHBI correctly identifies severe AMD within these regions.

Solid Energy, New Zealand's largest coal mining company, has recently recognised environmental management, including issues surrounding water quality, as a core business activity (PCE 2006). Incorporating biological monitoring into stream assessment and using biotic indices such as the pHBI would assist Solid Energy in assessing the impacts of AMD on in-stream flora and fauna, and in communicating results to the public. It would be useful to compare the effectiveness of the pHBI against the AMDI, a recently developed macroinvertebrate biotic index (Gray and Harding in press) in assessing AMD impact. Feio et al. (2007) found that macroinvertebrates and diatoms provided complementary information in stream health assessment; macroinvertebrates were more sensitive to changes in stream morphology, and diatoms to water chemistry. However, both groups have been shown to vary significantly with pH (Lewis et al. 2007) and metal concentrations (Hirst et al. 2002). Due to differences in generation time between diatoms and macroinvertebrates, the pHBI may respond to short-term changes in mine discharge, and the AMDI to long-term changes.

Overall, my results suggest that diatoms can be used in monitoring AMD remediation and degradation on the West Coast of the South Island. Diatoms are relatively easy and inexpensive to collect, and with little additional effort could be incorporated into existing monitoring plans. This study will hopefully provide an impetus for a more extensive trialling of diatoms as bioindicators in New Zealand streams.

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Appendix A

Water chemistry and GPS coordinates of 39 streams sampled between January and April 2011. Cond. = specific conductivity, I.H. = percentage iron hydroxide cover, DOC = dissolved organic carbon, Ref. C = circum-neutral reference, and Ref. NA = naturally acidic reference. DOC, Al, Fe, Zn, and Mn are in mg/L and conductivity is in μS_{25}/cm.

Stream #: name	Category	Easting	Northing	pH	Cond.	Al	Fe	Zn	Mn	I.H.	DOC
1: Lankeys	Ref. C	2419158	5895098	6.4	72	0.05	0.05	0.03	0.00	0	1.81
2: Burkes	Ref. C	2417654	5899492	6.8	46	0.18	0.17	0.04	0.01	0	1.91
5: Murray Trib 2	Ref. C	2420157	5896944	6.4	44	0.09	0.08	0.02	0.00	0	4.86
8: Italian	Ref. C	2420167	5906007	6.1	38	0.05	0.19	0.02	0.02	0	1.30
9: Coal	Ref. C	2420527	5905486	6.5	54	0.05	0.33	0.06	0.02	0	1.72
12: Burke Seddon.	Ref. C	2427966	5960351	6.4	42	0.21	0.05	0.04	0.00	0	1.30
13: Coal Seddon.	Ref. C	2427036	5959313	6.9	89	0.09	0.06	0.03	0.01	0	2.13
14: Chasm	Ref. C	2426286	5957971	6.7	52	0.10	0.06	0.02	0.00	0	1.70
22: Strongman Mine	Ref. C	2367569	5871390	7.7	203	0.09	0.65	0.02	0.02	0	3.27
23: Madman	Ref. C	2418277	5900914	6.4	63	0.25	0.69	0.02	0.02	0	6.51
24: Carldiers	Ref. C	2415149	5896665	5.9	32	0.20	0.27	0.02	0.01	0	5.17
25: Slab Hutt	Ref. C	2410602	5894614	6.0	33	0.23	0.33	0.01	0.01	0	5.43
31: Kiwi	Ref. C	2407368	5943628	6.0	76	0.10	0.13	0.04	0.00	0	4.10
37: Fuchsia	Ref. C	2400648	5929294	7.0	65	0.02	0.01	0.01	0.00	0	1.15
38: Omanu	Ref. C	2394919	5930668	7.2	42	0.07	0.04	0.02	0.00	0	2.93
39: Deadmans	Ref. C	2400744	5939367	5.8	51	0.19	0.50	0.03	0.03	0	3.84
3: Dauntless	Ref. NA	2419751	5902504	4.0	51	1.75	0.25	0.06	0.01	0	3.89

Stream #: name	Category	Easting	Northing	pH	Cond.	Al	Fe	Zn	Mn	I.H.	DOC
10: Rapid Trib	Ref. NA	2408347	5939680	4.1	22	0.16	0.13	0.03	0.00	0	2.48
21: Conns	Ref. NA	2409270	5940278	4.4	25	0.15	0.18	0.02	0.00	0	12.31
26: Tawhai	Ref. NA	2412119	5895106	5.7	30	0.28	0.36	0.02	0.01	0	6.00
4: Murray Trib 3	Moderate	2420311	5897078	5.8	43	0.07	0.42	0.02	0.05	97	2.42
6: Murray Trib	Moderate	2419893	5896527	3.4	48	0.68	0.58	0.05	0.09	100	1.42
7: Murray Main	Moderate	2419522	5896415	3.6	54	0.16	0.24	0.05	0.08	80	1.57
11: Page	Moderate	2424842	5961959	4.5	247	1.81	0.54	0.07	0.11	100	5.09
15: Wearne	Moderate	2422384	5957209	3.5	313	3.87	1.87	0.11	0.14	100	3.46
16: Twins	Moderate	2414061	5951493	4.6	179	0.12	0.07	0.04	0.03	65	0.96
20: Rapid	Moderate	2408031	5939187	3.5	125	9.98	18.91	0.55	0.31	80	0.50
36: Mine No. 1 Trib	Moderate	2417027	5952191	5.0	273	1.80	1.06	0.17	0.35	100	1.53
17: Granity	Severe	2414662	5952539	2.8	931	32.95	8.52	0.76	0.67	20	5.00
18: Millers	Severe	2416185	5951842	2.8	1078	41.37	11.39	0.96	0.83	25	1.27
19: Mine Lower	Severe	2417167	5954519	2.9	979	39.24	12.82	0.94	0.84	70	1.37
27: Bath House	Severe	2416108	5951699	2.1	1871	38.38	11.16	2.20	1.99	0	0.97
28: Hot Stream	Severe	2416056	5951582	2.1	1919	37.96	10.70	1.70	2.06	0	1.57
29: Mine Portal	Severe	2415950	5951548	2.1	1534	59.16	8.82	1.47	1.61	0	1.43
30: Pack Track	Severe	2416191	5951653	3.3	1248	32.57	10.55	1.29	1.44	0	1.76
32: Upper Miller	Severe	2416339	5951497	3.2	984	21.14	6.50	0.66	0.49	0	1.46
33: Warm Waterfall	Severe	2416749	5951587	3.1	1275	36.03	8.32	1.05	1.27	7	1.20
34: Old Dip Adit	Severe	2416353	5951517	3.2	1444	29.56	11.43	1.24	1.42	0	0.93
35: Mine No. 1	Severe	2417038	5952196	3.1	1726	29.85	7.56	1.56	1.90	52	0.81

Appendix B

Diatom taxa and relative abundance (%) sampled in 39 streams on the West Coast, South Island, New Zealand between January and April 2011.

Taxa	**Site** (% relative abundance)
Achnanthes cf. *petersenii* Hustedt	**4** (7.75), **15** (2.5)
Achnanthidium minutissimum (Kützing) Czarnecki	**1** (13.25), **5** (0.25), **9** (0.75), **12** (1.5), **13** (3.75), **14** (0.75), **22** (36.25), **23** (2), **24** (0.25), **37** (1), **39** (1), **26** (0.5), **4** (1), **11** (0.25), **15** (6.5), **16** (1.75), **34** (0.25)
Actinella aotearoaia R. Lowe, B.J.F. Biggs & R. Francoeur	**3** (0.25)
Adlafia bryophila (J.B. Petersen) Gerd Moser, Lange-Bertalot & D. Metzeltin	**20** (1.5), **21** (1),
Adlafia cf. *muscora* (Kociolek & Rivers) Gerd Moser, Lange-Bertalot & Metzeltin	**21** (1)
Amphora sp.	**20** (1.25)
Brachysira brebissonii R. Ross	**28** (0.25), **3** (0.25), **10** (2), **21** (2.75), **24** (2.75), **11** (1.25), **15** (7), **20** (3.5)
Brachysira serians var. *acuta* (Hustedt) P.B. Hamilton	**21** (0.5)
Cocconeis placentula Ehrenberg	**1** (0.5), **2** (0.75), **8** (0.25), **12** (5), **13** (2.25), **14** (32.25), **25** (1.75), **31** (53), **37** (47), **38** (0.25), **39** (0.25), **21** (0.25), **6** (0.5), **7** (0.25), **15** (2), **16** (26), **20** (0.25), **36** (0.25), **34** (0.5)
Craticula ambigua (Ehrenberg) D.G. Mann	**11** (0.5)
Cymbella aspera (Ehrenberg) Cleve	**23** (0.25), **4** (0.25)
Cymbella kappii (Cholnoky) Cholnoky	**1** (0.25), **2** (33.75), **12** (24.45), **13** (1.75), **14** (0.75), **16** (0.25)
Cymbella tumida (Brébisson) van Heurck	**31** (0.35)

Taxa	**Site** (% relative abundance)
Cymbelloid sp.	**5** (0.25)
Diatoma mesodon (Ehrenberg) Kützing	**1** (0.25), **2** (2.5), **5** (8), **9** (16), **12** (0.75), **13** (18.5), **22** (1.5), **24** (0.5), **37** (4.75), **38** (40.5), **3** (0.5), **20** (1.75)
Diatoma tenuis Agardh	**22** (11.25), **6** (1)
Diploneis elliptica (Kützing) Cleve	**15** (0.25)
Encyonema minutum (Hilse) D.G. Mann	**1** (1.5), **2** (0.5), **9** (3.75), **12** (4.5), **13** (24), **22** (0.75), **23** (1), **24** (1), **25** (18), **38** (19.5), **39** (0.5), **26** (18.75), **7** (0.5), **11** (0.25), **15** (9), **16** (1.25)
Encyonema prostratum (Berkeley) Kützing	**22** (1)
Encyonema silesiacum (Bleisch) D.G. Mann	**11** (0.25)
Encyonopsis microcephala (Grunlow) Krammer	**22** (0.5)
Epithemia adnata (Küzing) Brébisson	**14** (1.75), **16** (0.25)
Epithemia sorex Kützing	**2** (0.25)
Eunotia arcus Ehrenberg	**20** (0.25)
Eunotia bilunaris (Ehrenberg) Schaarschmidt	**23** (0.25), **25** (0.25), **39** (0.25), **3** (41.75), **10** (0.4), **21** (12.75), **26** (0.25), **6** (71.75), **7** (19), **20** (9) **18** (0.25)
Eunotia exigua (Brébisson ex Kützing) Rabenhorst	**23** (0.75), **24** (1), **25** (2), **38** (0.75), **39** (0.25), **3** (5), **21** (1.75), **26** (57.25), **24** (60.75), **6** (7.5), **7** (62.25), **11** (56.25), **15** (5.75), **16** (7.75), **20** (6.25), **36** (91.25) **18** (0.25)
Eunotia implicata Nörpel & Lange-bertalot	**37** (2.5), **38** (1.25), **4** (1.25), **11** (1.75), **15** (4.5), **20** (0.75)
Eunotia cf. *incisa* Gregory	**10** (51.5), **21** (4.75), **20** (36.25)
Eunotia minor (Kützing) Grunow	**5** (0.5), **14** (0.25), **24** (1.75), **25** (0.25), **31** (0.75), **3** (29), **10** (3.5), **21** (11.25), **26** (0.75), **11** (0.25), **20** (8.25), **35** (0.5)

Taxa	**Site** (% relative abundance)
Eunotia muscicola var. *tridentula* Nörpel & Lange-Bertalot	**24** (0.75), **25** (0.5), **31** (0.5), **39** (0.5), **26** (9.75), **11** (1.25), **15** (0.5), **16** (0.5)
Fragilaria bidens Heiberg	**37** (0.25)
Fragilaria capucina Desmaziéres	**1** (0.25), **2** (1), **9** (0.5), **14** (0.25), **23** (30.25), **31** (0.75), **38** (0.25), **39** (0.5), **3** (0.25), **15** (6), **16** (5)
Fragilaria capucina var. *capitellata* (Grunow) Lange-Bertalot	**9** (12.5), **12** (1.25), **13** (8.25), **14** (0.75), **23** (2.25), **25** (0.5), **37** (0.75), **38** (1.25)
Fragilaria capucina var. *distans* (Grunow) Boye-Petersen	**15** (4.75)
Fragilaria capucina var. *vaucheriae* (Kützing) Lange-Bertalot	**1** (1.25), **2** (10.5), **8** (3), **9** (10), **12** (2), **14** (1.25), **22** (2.25), **23** (0.5), **24** (1.25), **25** (6.25), **37** (0.25), **38** (5.25), **39** (2), **10** (0.25), **4** (0.75), **6** (0.25), **11** (0.25), **15** (0.25), **16** (0.25)
Fragilaria sp.	**10** (0.25)
Fragilariforma virescens (Ralfs) D.M. Williams & Round	**24** (1.75), **25** (0.5), **26** (1.5), **11** (0.25)
Frustulia crassinerva (Brébisson) Lange-Bertalot & Krammer	**5** (0.5), **9** (0.25), **24** (0.5), **38** (1), **39** (1.5), **3** (7), **10** (10.5), **21** (21.25), **26** (0.25), **6** (1.25), **7** (2.25), **11** (1.25), 20 (6.5)
Frustulia rhomboides (Ehrenberg) De Toni	**8** (0.25), **14** (1.5), **25** (0.75), **37** (1.75), **10** (1.5), **21** (5.25), **15** (1.75), **20** (5.25), **35** (0.25)
Frustulia rhomboides var. *capitata* (Mayer) R.M. Patrick	**3** (8.25), **10** (3.25), **21** (13.75), **6** (1.25), **7** (2.25), **11** (0.75), **15** (0.75), **16** (1.25), **20** (3.25)
Frustulia saxonica Rabenhorst	**9** (1), **24** (6), **25** (3.75), **37** (1.25), **38** (1.5), **39** (3.75), **3** (0.25), **10** (21), **21** (12.5), **26** (1.25), **6** (1.25), **7** (3), **11** (2), **15** (10), **16** (0.75), **20** (4)
Frustulia sp.	**3** (0.5), **15** (0.75)
Frustulia vulgaris (Thwaites) De Toni	**8** (0.25), **9** (1.75), **13** (0.25), **22** (0.25), **24** (2), **37** (0.5), **38** (0.25), **39** (0.5), **10** (0.75), **4** (0.5), **11** (2.25), **15** (0.5), **20** (0.25), **36** (0.75)
Gomphonema acuminatum Ehrenberg	**6** (0.25)

Taxa	**Site** (% relative abundance)
Gomphonema angustatum (Kützing) Rabenhorst	**8** (0.5), **22** (0.5), **24** (5), **25** (0.25), **31** (0.25), **11** (0.5)
Gomphonema angustum C. Agardh	**1** (2), **2** (2.25), **8** (0.25), **12** (0.5), **14** (0.25)
Gomphonema clavatum Ehrenberg	**1** (3.25), **2** (0.5), **8** (0.25), **9** (0.5), **23** (0.25), **25** (0.25), **37** (10), **28** (0.5), **16** (0.25)
Gomphonema gracile Ehrenberg	**9** (1.25), **3** (1), **15** (3), **36** (0.75)
Gomphonema minutum (C. Agardh) C. Agardh	**1** (2.75), **2** (8.5), **5** (0.5), **8** (54), **9** (0.25), **12** (17), **13** (3.5), **14** (20.5), **22** (3.75), **24** (2.5), **37** (2.75), **39** (0.75)
Gomphonema parvulum (Kützing) Kützing	**1** (51.5), **2** (12.5), **5** (5.5), **8** (12.5), **9** (22.75), **12** (4), **13** (6.75), **14** (4.75), **22** (20.5), **23** (8.75), **24** (8.5), **25** (0.5), **31** (2), **37** (2.75), **38** (0.25), **39** (3.25), **3** (1.25), **4** (4.25), **6** (0.75), **7** (2.5), **11** (0.5), **15** (4.25), **16** (0.25), **36** (0.75)
Gomphonema sp. 1	**24** (0.25)
Gomphonema sp. 2	**1** (0.25)
Gomphonema truncatum var. *turgidum* (Ehrenberg) R.M. Patrick	**31** (0.25), **38** (0.5)
Hantzschia amphioxys (Ehrenberg) Grunow	**20** (0.75)
Hippodonta capitata (Ehrenberg) Lange-Bertalot, Metzeltin & Witkowski	**20** (0.25)
Karayevia oblongella (Østrup) M. Aboal	**1** (0.5), **2** (2.75), **5** (18.5), **8** (1.25), **9** (18.25), **12** (3.75), **13** (1), **14** (6.75), **22** (0.5), **23** (19), **24** (53.25), **25** (7.75), **31** (39.75), **37** (3.75), **38** (3.75), **39** (54.5), **3** (2.25), **26** (6.5), **4** (8.75), **6** (3.25), **7** (0.75), **11** (5.5), **15** (14.75), **16** (0.25), **36** (0.25), **19** (0.25), **32** (0.5)
Lemnicola hungarica (Grunow) F.E. Round & P.W. Basson	**16** (0.1)
Meridion circulare (Greville) C. Argardh	**24** (0.75), **25** (0.25)
Meridion circular var *constrictum* (Ralfs) Van Heurck	**25** (7.25)

Appendix B

Taxa	**Site** (% relative abundance)
Navicula cf. *angusta* Grunow	**2** (0.75), **12** (8), **14** (9.25), **24** (0.25), **25** (0.75), **38** (3.75)
Navicula capitoradiata Germain	**6** (0.25)
Navicula hambergii Hustedt	**31** (0.25)
Navicula lanceolata Ehrenberg	**2** (2), **13** (0.5), **22** (1.5), **24** (1), **39** (0.5), **16** (0.25)
Navicula cf. *mediocris* Krasske	**20** (1), **21** (10.5), **24** (0.25)
Navicula cf. *mollis* (W. Smith) Cleve	**22** (11.25)
Navicula radiosafallax Lange-Bertalot	**9** (2), **25** (1.5), **37** (1), **39** (0.25), **26** (0.5), **15** (2.5)
Navicula rhynchocephala Kützing	**9** (0.25), **25** (0.5), **39** (0.5), **15** (0.25)
Navicula cf. *soodensis* Krasske	**5** (0.5)
Navicula sp.	**37** (0.75)
Navicula tridentula Krasske	**20** (0.75)
Naviculoid sp. 1	**11** (0.25), **14** (4.25), **38** (0.25)
Naviculiod sp. 2	**5** (0.75)
Naviculoid sp. 3	**5** (0.5)
Naviculoid sp. 4	**20** (2.75)
Naviculoid sp. 5	**15** (1)
Neidium ampliatum (Ehrenberg) Krammer	**20** (0.5)
Nitzschia cf. *amabilis* Suzuki	**16** (0.5), **19** (0.5), **28** (0.25), **34** (0.75)
Nitzschia amphibia Grunow	**16** (1.5)
Nitzschia clausii Hantzsch	**9** (0.25), **11** (0.5), **37** (0.25)
Nitzschia dissipata (Kützing) Grunow	**1** (0.25), **2** (3), **8** (3.75), **12** (0.5), **13** (0.75), **22** (0.5), **15** (0.75)
Nitzschia filiformis (W.Smith) Hustedt	**22** (0.25), **39** (0.25)
Nitzschia inconspicua Grunow	**22** (1.25)
Nitzschia palea (Kützing) W. Smith	**1** (0.5), **2** (3.25), **22** (0.5), **24** (0.5), **6** (0.25), **11** (1)

Taxa	**Site** (% relative abundance)
Nitzschia paleaeformis Hustedt	**10** (0.25), **11** (7), **16** (0.75), **17** (30.75), **18** (8.25), **19** (6), **27** (14.5), **29** (0.5), **33** (0.5)
Nitzschia sp.	**37** (1)
Pinnularia cf. *acidophila* Hofmann & K. Krammer	**12** (0.75), **38** (0.025), **3** (0.25), **10** (0.25), **26** (0.75), **15** (2.25), **16** (45), **20** (2.75), **36** (4.25), **17** (69.25), **18** (91), **19** (92.35), **27** (85.5), **28** (99.75), **29** (99.5), **30** (100), **32** (99.25), **33** (99.5), **34** (98.5), **35** (99.25)
Pinnularia cf. *amabilis* K. Krammer	**9** (0.75), **13** (0.25), **24** (0.25), **39** (0.25), **21** (0.25), **26** (2), **6** (0.25), **11** (1), **15** (1.75), **16** (0.5), **20** (0.5)
Pinnularia brandelii Cleve	**14** (0.75)
Pinnularia divergentissima var. *minor* Krammer	**18** (0.25), **32** (0.25)
Pinnularia gibba Ehrenberg	**24** (0.5)
Pinnularia karelica Cleve	**20** (1.25)
Pinnulaira cf. *saprophila* Lange-Bertalot, Kobayasi & Krammer	**39** (0.25)
Pinnularia cf. *subcapitata* W. Gregory	**3** (1), **5** (0.25), **7** (1)
Pinnularia viridiformis Krammer	**15** (0.5), **21** (0.25)
Planothidium haynaldii (Schaarschmidt) Lange-Bertalot	**13** (5.25), **37** (0.25), **38** (1.25), **7** (0.25)
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	**1** (3.5), **2** (4.5), **5** (63), **8** (22.5), **9** (2.75), **12** (5.5), **13** (4.5), **14** (0.5), **23** (0.25), **24** (1.25), **4** (0.5), **6** (0.75), **7** (0.5), **16** (2)
Reimeria sinuata (Gregory) Kociolek & Stoermer	**13** (1.25), **22** (0.25), **37** (1.25)
Rhoicosphenia abbreviata (C. Agardh) Lange-Bertalot	**16** (2.75), **20** (0.25), **37** (1.25)
Rhopalodia gibba var. *minuta* Krammer	**14** (0.25)

Taxa	**Site** (% relative abundance)
Rossithidium lineare (Smith) Round & L. Bukhtiyarova	**1** (16.25), **2** (7.5), **5** (1), **8** (1), **9** (4.25), **12** (15.75), **13** (16.25), **14** (13.25), **22** (5.25), **24** (7.25), **25** (0.5), **31** (0.5), **37** (4.25), **38** (8.25), **39** (27.75), **7** (0.5), **15** (0.75), **16** (3.5)
Sellaphora bacillim (Ehrenberg) D.G. Mann	**15** (2), **20** (0.25), **21** (0.25)
Sellaphora pupula (Kützing) Mereschkovsky	**15** (0.5), **25** (0.25)
Sellaphora seminulum (Grunow) D.G. Mann	**6** (0.25)
Stauroneis anceps Ehrenberg	**8** (0.25), **24** (0.25)
Stauroneis kriegeri R.M. Patrick	**3** (0.25)
Stauroneis cf. *obtusa* N. Lagerstedt	**11** (0.25)
Stauroneis sp.	**20** (0.5)
Suriella angusta Kützing	**2** (3), **12** (5), **13** (0.75), **24** (0.25), **31** (0.25), **3** (0.5), **6** (0.25), **7** (5), **11** (14.75), **15** (0.25), **16** (1), **20** (0.25), **36** (1.75)
Synedra ulna (Nitzsch) Ehrenberg	**1** (2), **2** (0.25), **6** (0.25)
Tabellaria flocculosa (Roth) Kützing	**9** (0.25), **23** (31.75), **24** (0.25), **25** (46.75), **38** (3.25), **39** (0.75), **3** (0.5), **10** (1), **4** (1.75), **6** (0.25), **15** (2.5)
Ulnaria acus (Kützing) M. Aboal	**13** (0.5), **22** (0.25), **31** (1), **6** (0.25)
Ulnaira biceps (Kützing) P. Compère	**37** (0.25)
Ulnaria delicatissima (W.Smith) M. Aboal & P.C. Silva	**15** (0.25), **23** (2.75)